STUDIES ON THE GENETICS OF FRAGARIA

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INTRODUCTION

In 1894, A. MILLARDET, Professor of Botany at Bordeaux, published a rather extraordinary paper entitled "Note sur l'hybridation sans croisement ou fausse hybridation." In this report the author records the data obtained in a study of Fragaria crosses extending over a period of eleven years, which led him to the conclusion that species hybrids in this genus are not to be regarded as combinations of the two parental types, as in other forms, but that the characters either of the maternal or of the paternal parent are normally reproduced intact in the first and in all succeeding generations. MILLARDET's unusual results led Count SOLMS-LAUBACH, Professor of Botany at Strassburg, to undertake a further investigation of the genus. His results were conflicting, but he believed he was able to verify, in one cross at least, the occurrence of "hybridation sans croisement" as reported by MILLARDET.

As a result of these investigations the genus Fragaria has frequently been referred to in genetic literature as an exception to the usual rules of

inheritance. Since anomalous phenomena are a great stimulus to scientific progress, it seemed desirable to re-examine in detail the behavior of species crosses in this genus. Questions concerning the taxonomic relationship of the various species, the origin of the cultivated varieties and the expression and inheritance of sex gave to the material an added interest.

DESCRIPTION OF SPECIES AND VARIETIES USED

The following species and varieties of Fragaria have been used in these studies. With the exception of a few garden varieties sent to us by commercial firms, they were obtained from the UNITED STATES DEPARTMENT of AGRICULTURE through the kindness of G. M. DARROW and C. V. PIPER.

The chromosome counts were made in this laboratory by Mr. K. ICHIJIMA (1926).

The 7-chromosome group

F. vesca L. Wood strawberry. Fraisier des bois. Rootstock and runners rather slender. Petioles slender, with soft, spreading white hairs; leaflets almost sessile, light green; margin toothed along upper two-thirds, entire along lower third; scapes strong and erect, many flowered, usually overtopping the leaves; flowers small, hermaphroditic, petals concave;

FIGURE 1.—7-chromosome species. At left, *F. vesca* (European); at right, *F. bracteata* (American).

calyx lobes spreading or reflexed; stamens hardly as tall as receptacles; styles very numerous, short and relatively thick; fruit roundish or conical, detaching easily, sweet but lacking in the acid flavor characteristic of *F. virginiana;* flesh soft and pulpy or mealy; achenes set superficially.

This species has an exceedingly wide range. It is the common wild strawberry of Europe. Forms indistinguishable from it have been reported from Ecuador and Peru, though they may have been introduced, from both Western and Eastern North America, and from Northern Asia.

F. vesca L. From Ecuador, indistinguishable from European form except in shape of berry, which is almost spherical.

F. mexicana Schlecht. Obtained by the UNITED STATES DEPARTMENT of AGRICULTURE from the BOTANICAL GARDEN of BOHEMIA. It appears to be an alpine form of *F. vesca*, presumably introduced into Mexico, and escaped from cultivation. It is difficult to distinguish from *F. vesca;* very free blooming, berries long and conical.

F. vesca Rostrup. A pink-flowered *vesca*.

F. americana alba (Porter). The appearance of the plant is very similar to that of *F. vesca*, though the petioles are somewhat shorter, scapes shorter and weaker. The fruit is white or creamy and the seeds straw-colored.

F. bracteata Heller. Similar to *F. vesca* but the leaves are smaller and the petioles shorter. The growth habit is almost prostrate; petioles and peduncles densely pubescent; scape weak and few flowered; styles short, recurved and few in number; stamens long, overtopping receptacle; fruit spherical, soft and pulpy; achenes slightly sunken in shallow pits. *F. bracteata* ranges from New Mexico to Idaho and Washington.

FIGURE 2.—21-chromosome species, *F. elatior*.

F. californica Cham. & Schlecht. California wood strawberry. The growth habit is scarcely distinguishable from that of *F. vesca;* petals very large, thin and finely veined; pistils short, recurved and few in number; stamens long, towering above receptacle; the fruit small, spherical; pulp soft and juicy; achenes slightly sunken in shallow pits. Similar to *bracteata* except in growth habit. Pacific Coast.

The 21-chromosome group

F. elatior Ehr. (*F. moschata* Duchesne). Hautbois strawberry. Similar to *F. vesca* in appearance but leaves, petioles and peduncles heavily pubescent; leaves strongly veined and pale green; flowers large, usually dicœcious; styles rather short and thick; petals thick waxy; calyx reflexed; fruit long conical, light red, sweet and with a slightly musky flavor. The range of *F. elatior* includes Central Europe, Sweden, Finland and parts of Russia. Two hermaphroditic garden varieties of this species, Monstreuse Hautbois and Black Hautbois, have been used in these investigations.

The 28-chromosome group

F. virginiana Duchesne. Scarlet or Virginia Strawberry. Rootstock moderately stout; runners long and rather stout; petioles intermediate between *F. vesca* and *F. chiloensis;* leaflets usually stalked, firm in texture and dark green in color; scape about as long as petioles, many flowered, in some forms strong and erect, in others weak and prostrate; usually dicœcious; stamens on male flowers long and numerous; calyx often fails to open completely, remaining half closed after petals fall; fruit spherical to short conical, usually with a smooth neck, aromatic and highly flavored; color a bright scarlet; flesh moderately firm; achenes sunk in deep pits. Found in a wide variety of types throughout Eastern North America.

F. virginiana No. 27. An erect, robust, free-flowering pistillate form.

F. glauca Watson. A form of *F. virginiana* from Canada. Scapes strong and many flowered; pubescence scanty throughout.

F. chiloensis L. Chilean strawberry. Rootstock stout, runners strong; petioles stout, densely hairy in some forms, almost glabrous in others; leaflets more or less stalked, thick, leathery and more or less glossy dark green above; pale and quite villous and strongly veined underneath; leaflets wide in proportion to length; outer third crenate with short, rounded teeth, terminal one small; scape usually shorter than leaves, weak, usually few flowered; shape of petals variable in different races; calyx closing after petals fall; usually dicœcious; styles rather short; stamens in male flowers long and numerous; fruit pale pink to dull red, more or less translucent, almost spherical, very aromatic; flesh firm; achenes superficial or sunk in shallow pits. This species includes many types which grade into one another. Its range extends from Patagonia to Alaska. A pistillate clone received under the name Oregon No. 2 has been used most extensively in crosses. It agrees with the descriptions of *F. cuneifolia*, a subspecies of *F. chiloensis*.

F. grandiflora Ehr. Common garden strawberry. Believed to have originated from crosses between *F. virginiana* and *F. chiloensis*. Extremely variable, some forms leaning toward *virginiana*, some toward *chiloensis*. In most characters more or less intermediate, but in size of plant, flower and fruit surpassing that of either in our specimens. Flowers often with supernumerary petals. A number of cultivated varieties have been kept under observation.

Species from other genera used

In addition to the Fragaria species enumerated the following two forms have been used in crosses:

Duchesnea indica Andr. Mock or Indian strawberry. Petioles low, spreading; leaves trifoliate, dark green; runners bearing leaves and one-flowered peduncles; petals yellow; bractlets large and dentate at tips; fruit spherical, bright red, watery and insipid; achenes very prominent. Introduced from Northern India. Chromosome counts by ICHIJIMA show 42 chromosomes.

FIGURE 3.—28-chromosome species. At left, *F. virginiana;* at right, *F. chiloensis.*

Potentilla nepalensis hybrid. Horticultural variety Miss Willmott. Plant erect, branching; leaves 5-foliate clothed with long, soft spreading hairs; petals, stamens and pistils rose colored to dark red; pollen bright yellow; receptacle dry and hairy when mature; achenes small. Introduced from the Himalayas. Chromosome number undetermined.

TECHNIQUE USED IN MAKING CROSSES

Emasculation, though tedious because of the large number of anthers, is not difficult. A pair of straight dissecting forceps, the tips of which had been ground down to a fine point, have proved to be best suited to the purpose. At the beginning of the investigation the buds were emasculated

immediately before opening. At this stage the work can be done quite rapidly and with a minimum of injury to the receptacle. But though dehiscence does not occur until several hours after the flower has opened, there appears to be some danger when emasculation is done at this stage of functional pollen grains being expressed from the anthers when they are grasped by the forceps, and recently the practice of emasculating two to four days before opening has been followed. This age represents a compromise between opposing considerations; a younger age might be still safer, but the percentage of failure due to the injurious effect of exposing the young pistils is extremely high.

All open flowers and all buds except those to be used, are removed before emasculation and the plant is subjected to a thorough spraying under a stiff stream of water to remove any pollen which may be adhering to the leaves.

Bagging the individual flowers after emasculation proved unsatisfactory, conditions within the paraffin bag apparently being unfavorable to development. Accordingly the plan of removing the emasculated plants to a house containing no other Fragaria individuals was adopted. The plants are set upon inverted saucers placed in the center of a pan of water to isolate them from ants, which visit the flowers diligently. The ventilators are covered with mosquito netting, and flower-visiting insects seldom find their way into the greenhouses, but the plants are covered with wire cages as an additional precaution. Whenever possible the crossing is so planned that only a single kind of pollen is used at a given time. When crosses involving several kinds of pollen are made simultaneously, the plants are removed individually to a second isolation house for pollination in order to guard against pollen being wafted to plants destined for crossing with another species.

The earlier crosses were made without using all of these various precautions, which were adopted only as the extreme difficulty of avoiding contamination became more and more evident. The pollen grains are extremely minute (about 15–20 micra in diameter) and apparently they are readily picked up and carried about by air currents. The extent of the danger of contamination by ants has been recognized only recently. It appears that these insects travel considerable distances from one plant to another, their objective being always the open flowers from which they obtain nectar.

The seeds apparently require an after-ripening period of several months. If sown immediately after they have completed their development, germination is irregular and often is considerably delayed. Scarify-

ing the seeds by rubbing them over emery cloth or fine sandpaper appears to hasten germination, presumably by facilitating the absorption of moisture. Sterilized soil is used in the seedpans, this as a safeguard both against "damping-off" and against the danger of introducing foreign Fragaria seed with the soil.

There is considerable variability in the length of time required by the different species to reach maturity. The 7-chromosome group matures most rapidly, occasional individuals having bloomed at the age of five to six months. The 28-chromosome group requires a longer time, usually from seven to ten months. The 21-chromosome species, *F. elatior*, is the slowest to develop. It seldom blooms before the end of the first year. In all the species backward individuals are found in which blooming may be delayed for several years.

Sowings may be made at any season. Those made in the spring and summer months succeed somewhat better than do those made during the fall and winter, but the difference is not great.

DESCRIPTION OF CROSSES

Species of the 7-chromosome group crossed inter se

The species belonging to this group are all similar to each other morphologically, as well as identical in their chromosome numbers, so it was to be expected that they would cross readily with each other and would give fertile hybrids. Since the expected does not always happen in genetical work, however, the crosses listed below were made for the purpose of determining the facts. In all of the crosses listed, the female parent is mentioned first.

F. californica×*F. bracteata*. F_1 plants vigorous. Ten plants were grown. The three which have thus far bloomed have proved to be fully fertile.

F. californica×*F. vesca rosea*. F_1 plants normal and fairly vigorous. Ten F_1 plants were grown. The two which have bloomed have proved to be pink-flowered, as expected, and were fully fertile.

F. mexicana×*F. americana alba*. Ten F_1 plants were grown. All were normal and moderately vigorous. The seven which have bloomed were red-fruited and fully fertile.

F. americana alba×*F. mexicana*. Ten F_1 plants were grown. All were quite normal and vigorous. The six which have bloomed were fully fertile and were red-fruited.

F. vesca rosea×*americana alba*. Ten F_1 plants were grown. They were exceptionally vigorous. The seven which bloomed were fully fertile and were red-fruited and pink-flowered.

F. vesca rosea×*F. mexicana*. Ten F_1 plants were grown. All were vigorous. The six which bloomed were pink-flowered and fully fertile.

F. vesca (Ecuador)×*F. vesca rosea*. Ten plants were grown. All were vigorous and fully fertile. The pink flower of the *rosea* parent was known to be dominant to white flower. It was expected, therefore, that all of the F_1 plants would prove to be pink-flowered; three, however, were white-flowered. This result could have come about in one of several possible ways; namely, accidental selfing, heterozygosity of the pink-flowered parent, or pseudogamy.[1] The identical *rosea* parent was not retained and it is not known which of these causes was responsible for the appearance of the white-flowered individuals.

F. vesca (Ecuador)×*F. americana alba*. Ten F_1 plants were grown. All were vigorous. The three which have flowered have proved to be red-fruited and fully fertile.

F. vesca (Tiflis, Russia)×*F. vesca rosea*. Ten F_1 plants were grown. All were vigorous. The seven which have flowered were red-fruited and fully fertile.

F. bracteata×*F. vesca rosea*. Ten F_1 plants were grown. They were only moderately vigorous. Six were pink-flowered and four white-flowered. In this case it is fairly certain that the *rosea* parent was heterozygous for pink flower, since the white-flowered plants were intermediate between the two parents in growth habit.

F. bracteata×*americana alba*. Ten F_1 plants were grown. They were vigorous and intermediate between the two parents in growth habit. All bloomed, and proved to be red-fruited and fully fertile.

As was to have been expected from the identity of their chromosome numbers and from their morphological similarities, all of the 7-chromosome species were found to cross readily with each other and to give vigorous fertile progenies. Where discernible differences existed between the two parents the F_1 plants were intermediate, except in flower color and fruit color, pink flower color being dominant to white and red fruit color to white.

7-chromosome species×*21-chromosome species*

F. vesca×*F. elatior* (Black Hautbois). A number of crosses were made and all set fruit quite readily. The resulting seeds were apparently plump and well developed. Over 400 were sown; of these only three germinated. The seedlings were extremely weak, failed to develop a normal green color

[1] Pseudogamy is here used to mean the production of seeds by apogamy or parthenogenesis as a result of the stimulus of pollination.

in the cotyledons, and died within one to two weeks after germinating without having developed a single leaf. A second sowing of 200 seeds resulted in only 1 seedling which also perished at the age of about two days.

This result was unexpected because *F. vesca* and *F. elatior* resemble each other fairly closely in general appearance, and especially in the conformation of the fruit. It is all the more surprising, because crosses between the 7-chromosome and the 28-chromosome groups, which differ quite profoundly from each other in morphological characters in addition to the greater discrepancy of their chromosome numbers, give viable F_1 plants.

It is impossible with our present meager knowledge to hazard even a guess as to the reasons for the extreme incompatibility between these two forms. FOCKE (1881) cites KNIGHT as authority for the statement that WILLIAMS succeeded in producing a hybrid from the reciprocal cross, *F. elatior*×*F. vesca*. Our attempts at making the cross in this direction have resulted without exception in failure, not a single flower setting fruit.

7-chromosome species×*28-chromosome species*

F. vesca×*F. chiloensis*. Flowers of *F. vesca* pollinated with *F. chiloensis* pollen set fruit freely, but a large percentage of the resulting seeds is small and poorly developed. Even the plump seeds germinate poorly, a total of over 1200 fairly well developed seeds producing only 6 seedlings. By the time the latter were a month old it began to be apparent that the uniformity usually obtained in the F_1 generation of species crosses did not exist here. Four of the plants were extremely weak, and produced miniature leaves of a yellowish green color, while two were quite vigorous. As the plants grew older the discrepancy between them increased. The weak individuals failed to improve, their new leaves showing but little increase in size and no improvement in color. The two vigorous plants grew rapidly and in the course of six months attained a size equal to that of their parents. The dark bluish-green color and the thick texture of the *chiloensis* leaf is dominant over the thin, light green character of the *vesca* leaf. The plants resemble the *chiloensis* parent much more closely than *vesca*, although their relatively upright growth habit suggests the *vesca* parent. Neither plant has bloomed. Two of the dwarfs have died, the two which remain showing but little growth. Their leaflets are scarcely more than a centimeter long, while those of their normal sisters are 4–5 centimeters long. Their abnormal color and their minuteness make it

difficult to see any resemblance to either of the parents. Possibly they are haploids, but their poorly developed root system has made it impossible to get chromosome counts.

F. americana alba×*F. glauca*. Flowers of *F. americana alba* set fruit quite readily when pollinated by *F. glauca*, but a large proportion of the achenes remain undeveloped. Even those which appear to be normal, germinate poorly. Only one plant was obtained from a sowing of 66 seeds. It is conspicuously lacking in vigor. Its leaves are extremely small, about

FIGURE 4.—*F. vesca*×*F. virginiana*, F_1. Above, vigorous but sterile hybrid; below, two dwarf hybrids.

one-fifth to one-tenth the size of those of the parents. Their relatively thick texture suggests the influence of *F. glauca*. In shape and general appearance they display no particular resemblance to either parent. The plant has never produced runners, nor has it bloomed, although it is now over two years old.

F. vesca×*F. glauca*. Flowers of *F. vesca* pollinated with pollen from *F. glauca* set fruit readily, but as in the previous cross many of the achenes were poorly developed, and even the plump achenes germinated poorly. A sowing of 250 apparently normal achenes gave only 5 plants. One of the plants proved to be exactly like the female parent. It must have resulted either from pseudogamy or from accidental selfing. The other 4 are obviously hybrids. All are lacking in vigor. As in the *vesca-chiloensis* cross there is a conspicuous lack of uniformity in the population. Three of the plants are too small and weak to show any definite resemblance to either parent, and have never bloomed. The fourth plant is somewhat more vigorous, but still is considerably less vigorous than either parent. In color and texture the leaves resemble the *glauca* parent. It has bloomed rather freely but the flowers are small and abnormal in appearance. The petals are greenish; the stamens are rudimentary and never produce pollen. Numerous pollinations with pollen from the two parents have been without effect. Apparently the pistils are completely sterile.

F. vesca rosea×*F. glauca*. This cross may be made without difficulty but as in the previous case many of the resulting seeds were poorly developed. Germination of the plump seeds was only fair; a sowing of 76 seeds gave only 7 plants. Of these 5 are weak dwarfs with miniature leaves, which differ considerably among themselves. One produces a very large number of extremely minute leaves, giving the plant a fernlike appearance. None of these dwarfs has produced runners or flowers. The sixth plant is somewhat more vigorous; it produces many runners but it has never bloomed. The remaining plant, though undersized, produces flowers freely. They are very small, the anthers are rudimentary and produce no pollen, and the pistils have proved to be sterile with pollen from each parent. The color and texture of the leaves of this plant suggest the *glauca* parent.

F. vesca×*F. virginiana*. This cross regularly results in fruit setting, but many of the achenes remain small. Even those which appear plump and well developed, germinate poorly. From 500 seeds only 22 plants were obtained. Here again the F_1 population was extremely variable. Of the 22 plants 1 proved to be a pure *vesca*, like the female parent; whether it resulted from pseudogamy or accidental selfing is unknown. Eleven were miniature dwarfs. Several have died, the remainder are kept alive only with difficulty. They show considerable variability in color and leaf shape. None of these dwarfs has bloomed or produced runners. The remaining 10 plants are quite vigorous. They bloom freely but the flowers are small with rudimentary anthers which are quite sterile.

Pistil sterility also appears to be complete. A number of pollinations have been made with pollen from each parent without success. These 10 plants all resemble the *F. virginiana* parent quite closely, the dominance of the dark green color and leathery texture of the leaves as found in *F. virginiana* being particularly noticeable.

F. bracteata×*F. virginiana*. Fruit setting results quite regularly from this cross, but the number of seeds per fruit is small. A sowing of 24 seeds yielded 12 plants. Ten are true hybrids which resemble the *virginiana* parent much more closely than the *bracteata* parent, although there is a clear resemblance in leaf shape to the latter. The flowers are well developed and the anthers are almost normal in appearance. Occasionally a small amount of pollen is produced, which is mostly shrunken and abortive, though some grains appear to be normal. Selfing and backcrossing have thus far resulted in failure.

FIGURE 5.—*F. vesca*×*F. chiloensis*, F_1. At left, vigorous hybrid; at right, dwarf hybrid.

The other 2 plants present a most peculiar case. They are identical with the *bracteata* parent in appearance except that the flowers have no anthers; they are pistillate, whereas *F. bracteata* is hermaphroditic. True, isolated pistillate flowers have been observed on *F. bracteata* which may be the result either of fluctuation or of mutation, but they are rare. If these two plants are not true hybrids, they are the only purely pistillate individuals thus far encountered in the 7-chromosome group of species. Except for the anomalous pistillate flowers sometimes found on *F. bracteata* the group is hermaphroditic.

These two pistillates were crossed with *F. vesca rosea*. The progeny all proved to be hermaphroditic. It follows that the failure to develop stamens, if genetic, is due to the action of a recessive factor. The F_1 plants have been selfed but F_2 plants have not yet been grown.

Flowers of the 7-chromosome forms set fruit quite readily when pollinated with pollen from the 28-chromosome species, but many of the resulting achenes are poorly developed and even those which appear normal germinate poorly. Reciprocal crosses all resulted in failures.

The resulting F_1 populations are extremely variable. Three fairly distinct types may be recognized:

1. Vigorous hybrids, usually showing more or less complete dominance of the 28-chromosome type, with completely sterile flowers.

2. Weak miniatures and dwarfs, quite variable in appearance among themselves, which never flower.

3. Individuals of the maternal type. These appear only rarely. Whether they result from pseudogamy or from accidental selfing is not known.

Individuals intermediate between the first and second types are also occasionally found.

The 7-chromosome species crossed *inter se* give F_1 plants which show minor variations; the same is true of the 28-chromosome species. A possible explanation for the extreme variability of the hybrids between these two groups is that the variation in the gametes of the parental species resulting from their slight heterozygosity is enormously magnified in the F_1 zygote. Differences which are relatively insignificant in the economy of the pure species assume great importance in the hybrids. UPHOF (1922) reports similar F_1 variability in crosses between *Phaseolus vulgaris* and *Phaseolus multiflorus*. Since the variability is here even more difficult to explain because the two parental species are naturally self-fertilized and should therefore be quite homozygous, it is possible that in both cases chromosomal differences resulting from an elimination of one or more chromosomes in early zygotic divisions may be responsible for the differences in appearance.

Of these two explanations for the phenomenon as found in Fragaria, the first seems more plausible. A bit of evidence which appears to favor it is the following: *F. virginiana* and *F. glauca* are scarcely distinguishable from each other, and when crossed with other 28-chromosome species they usually give similar progenies; but there is a great difference between the F_1 populations which they produce when crossed with *F. vesca*. The former gives a certain percentage of vigorous, normal-appearing hybrids, while the latter has never given hybrids approaching either parent in robustness. Possibly differences exist between the two which, though of minor importance in crosses with other 28-chromosome species, are of great significance in hybrids between the two chromosome groups.

The possibility also remains that some of the dwarfs produced are haploids.

28-chromosome group × 21-chromosome group

F. virginiana×*F. elatior*. A pistillate form of *F. virginiana* was used as the female parent. It set fruit readily when pollinated with pollen from *F. elatior*. Most of the achenes were well developed and showed a high germination percentage, 100 seeds giving 90 plants. Here again the F_1 population was not uniform, although the variability was not nearly so great as in crosses between the 7-chromosome and 28-chromosome species. Some plants were very vigorous, others were relatively weak, with many

FIGURE 6.—*F. virginiana*×*F. elatior*, F_1. Practically indistinguishable from *F. elatior*, but sterile.

gradations between the two extremes. The differences, however, instead of becoming more pronounced as the plants grew older, gradually diminished, and at the present time the population appears to be fairly uniform.

The dominance of the *elatior* type is striking. Many of the F_1 plants can scarcely be distinguished from the pure species. Its dense, shaggy pubescence, the rather pronounced venation of its leaves and the conformation of its flowers with their short styles and soft petals have been reproduced almost intact in the hybrids. Yet the hybrids appear to be completely sterile.

The dominant stamen-inhibiting factor, for which the female parent is heterozygous, apparently shows the same type of segregation in this cross as in intra-specific crosses, for though only a few plants have bloomed,

both the staminate and the pistillate forms are represented. In addition to the true hybrids, some 15 plants of the maternal type appeared. Since the female parent was a pistillate, selfing cannot have occurred. However, at the time the cross was made other plants in the same isolation house were being pollinated with *virginiana* pollen; it is barely possible that contamination may have resulted from this source. The cross has been repeated under conditions which preclude the possibility of contamination. If plants of the maternal type appear in the resulting progeny, the occurrence of pseudogamy will be definitely established.

The "maternals" include both staminates and pistillates. It follows that if they have indeed resulted from pseudogamy, parthenogenesis rather than apogamy is responsible for their appearance, because the latter would produce plants identical with the mother—, that is, pistillates. The pistillate individuals which appeared if produced parthenogenetically should be homozygous for the dominant stamen-inhibiting factor. Several

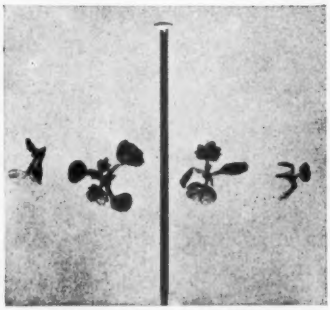

FIGURE 7.—*F. vesca*×*D. indica*, F_1. A common pin shows the size of these plants.

of them have been crossed with hermaphrodites to determine their constitution with respect to this factor. The outcome will answer the question as to their origin; if they prove to be heterozygous for the factor they must have resulted from contamination, if they are homozygous for the factor they must have been produced parthenogenetically.

Generic crosses

F. vesca (7 chromosomes)×*Duchesnea indica* (42 chromosomes). Flowers of *F. vesca* pollinated with pollen from *D. indica* almost invariably set fruit. Many of the achenes, however, fail to develop normally. Germination is fairly high, 500 seeds producing about 30 plants. Four of these plants were apparently pure *F. vesca* and must have resulted either

from accidental selfing or from pseudogamy. The remainder are evidently true hybrids. They are extremely small and weak. The leaves present a characteristic mottled appearance, due apparently to some abnormality in chlorophyll distribution. Many died before the end of the first month. In the course of that time they had developed as many as 15–20 miniature leaves. Four plants photographed at the age of five weeks are shown in figure 7.

F. vesca (7 chromosomes)×*Potentilla nepalensis* hybrid var. Miss Willmott (chromosome number unknown). This cross is difficult to make. Only one-third to one-half of the flowers pollinated set fruit, and each fruit bore only a few well developed achenes. A total of 141 achenes was obtained, which yielded two seedlings. Their color was a pale yellowish-green. In neither was there any indication of plumule development; both died within two weeks after germinating.

28-chromosome species crossed inter se.

The following crosses were made between species belonging to the 28-chromosome group:

F. chiloensis (Oregon No. 2)×*F. virginiana*

F. virginiana (pistillate)×*F. chiloensis* (Oregon No. 1)

F. virginiana (pistillate)×*F. glauca*

All of the above crosses are easily made. They gave in every case uniformly well developed achenes which showed a high germination percentage. The F_1 plants from all three crosses are vigorous, fairly uniform, fully fertile and intermediate between the two parents. The sex differences show normal segregation. They will be treated in another paper.

PARTHENOCARPY, PARTHENOGENESIS AND APOGAMY

During the summer of 1925 a group of pistillate plants of *F. virginiana* and *F. chiloensis* were isolated by placing them at the extreme end of one of the greenhouse benches. An assortment of plants of commercial varieties and of pure species were situated at the other end of the same bench some fifty feet away. The intervening space was filled with tall Nicotiana plants. In view of the absence of flower-visiting insects, the ventilators having been covered with mosquito netting, it was believed that the isolation was complete. During the months of March, April and May, these plants—all pistillates—developed no fruit except when artificially pollinated. In June, however, after a period of extremely hot weather, occasional seeds developed on flowers which, so far as was known,

were unpollinated. It was believed that the high temperature, which appeared to be the only variable, might have been instrumental in stimulating apogamic development.

To obtain further evidence on the question fifteen individuals, including the commercial varieties Haverland, Warfield, Sample and Kellogg's Prize and the species *F. chiloensis* and *F. virginiana*, were placed in a glass chamber, the temperature of which was maintained at 40°–45°C. Each plant bore from 20 to 50 flowers, but not one showed the slightest trace of achene or receptacle development.

HABERLANDT (1921) was able in *Oenothera Lamarckiana*, by wounding the ovules in various ways, to induce the development of what he considers to be adventitious embryos. He found both bruising the ovules and piercing them with a needle to be effective in inducing the formation of these embryo-like structures. A number of receptacles of the isolated pistillate Fragaria plants were treated in a similar fashion, but in no case did the treatment result in an increased development,—indeed the receptacles usually degenerated the more rapidly as a result of the treatment.

In addition to the plants kept at a high temperature in the glass chamber, 125 pistillate plants—including *F. chiloensis*, *F. virginiana* and the commercial varieties Haverland, Hunterdown, Paul Jones, Kellogg's Prize, Big Late, Warfield and Portia—were isolated in a separate house and kept at ordinary greenhouse temperatures. The plants produced an average of not less than 25 flowers each, making a total of about 3000 flowers under observation. The receptacles of five flowers of Haverland, two of Warfield and two of Kellogg's Prize developed into berries, which were entirely normal in shape, color and texture, though only about one-fourth the usual size. The development, however, was confined to the receptacle—none of the achenes showed any increase in size. What the variables may have been which induced these flowers to further development while contemporaneous flowers on the same plant remained undeveloped, is not known. The position of the flowers on the cyme appears to be immaterial, since primary, secondary and tertiary flowers were represented. Two other flowers, these on a *F. chiloensis* plant, showed an aberrant behavior. When the flowers opened small terminal buds could be seen at the tips of the receptacles. These buds eventually produced several leaves which, though small, were entirely normal in other respects. The receptacles also underwent a considerable amount of development; they became fleshy and, in due time, passed through the normal ripening process, developing the color and aroma characteristic of the species.

One of these fruits was allowed to remain on the plant; the receptacle became fully ripe and finally decayed, resulting in the death of the little plant at its tip which had by this time produced three good leaves and had attained a height of about 2 centimeters. On neither fruit had the achenes undergone development.

These parthenocarpic fruits are of no significance from the genetic standpoint, except that they indicate that certain individuals show a greater tendency in this direction than others. As stated, in none of the eleven parthenocarpic fruits did the achenes undergo development, nor was any significant increase in size of achenes found in any of the 3000 flowers examined. It appears that spontaneous development of achenes in the 28-chromosome group, if it occurs at all under greenhouse conditions, is rare. It cannot be said, however, that it may not be encountered under other conditions or in other species of Fragaria. It is quite possible, too, that parthenogenesis and apogamy, if they do occur, as the rather frequent appearance of maternal plants in progenies resulting from species crosses might seem to indicate, are brought about only through the stimulus of penetration by the pollen tubes of foreign species. On this point these observations throw no light.

The fact that the "spontaneous" setting of seed observed on similar plants during the summer of 1925 failed to occur under more carefully guarded conditions, leads to the conclusion that the setting then obtained must have resulted from pollen carried by ants or by air currents. The extent of the danger of contamination from these two sources was not fully recognized at the time. It becomes more and more evident as the work progresses that the ordinary precautions to prevent contamination which have been found to be effective in other plants are not to be relied upon in Fragaria.

To determine whether pollination by other Rosaceæ is effective in bringing about development of the receptacles or achenes, nine plants of *F. vesca* were isolated, all the available flowers (from 10 to 15 on each) were emasculated, and each plant was pollinated with one of the following kinds of pollen: *Exachorda*, *Prunus*, *Chaenomoles*, *Rubus*, *Malus*, *Pyrus*, *Potentilla sterilis*, *Potentilla nepalensis* hybrid (var. Miss Willmott) and *Duchesnea indica*. Only the last two produced any visible development of the receptacle; flowers pollinated by the remainder withered at about the same time as did the unpollinated checks.

All of the flowers pollinated by *Duchesnea* produced good berries each with ten to twenty well developed achenes. Pollinations by *Potentilla nepalensis* resulted in three berries, each with two to five good achenes.

In the latter case the development of the receptacles was confined to the regions occupied by the achenes, as a result the berries were quite irregular in shape.

A large number of pistillate flowers of *F. virginiana* and *F. chiloensis* have also been pollinated with pollen from *Potentilla sterilis*, *P. nepalensis* and *Duchesnea indica*. Only the latter has proved effective in bringing about the development of fruit. The achenes are comparable in size to those resulting from pollination by the same species. The seeds are not yet ripe enough to test for germination, but it is to be supposed that here true fertilization has taken place.

The occasional spontaneous occurrence of parthenocarpy independent of achene development suggested the possibility that pollination with foreign pollen might produce a similar result. In every case, however, receptacle development was restricted to the regions immediately surrounding fertilized achenes.

INHERITANCE OF FLOWER COLOR AND FRUIT COLOR IN *FRAGARIA VESCA*

There is a fairly wide range of variability in leaf shape, leaf texture, pubescence, flower type, fruit shape and growth habit in Fragaria; but all of these characters appear to depend upon the interaction of a large number of factors, giving in F_1 an intermediate type, and in F_2 a series of gradations to which only an arbitrary classification can be applied. Only two characters, flower color and fruit color, and these only in the 7-chromosome group of species, were found to be simple and definite enough in their expression that their inheritance could be followed accurately.

The results of MILLARDET (1894) had suggested that Fragaria might present an exception to the usual rules of inheritance, or that the possible presence of parthenogenesis or apogamy might be expected to result in disturbed ratios. A study of the inheritance of flower color and fruit color was, therefore, undertaken to determine whether these two characters are inherited in a regular fashion, and whether they are independent or linked.

Flower color. The pink-flowered *F. vesca* used in the crosses is presumably identical with the type named by ROSTRUP (1888) *F. vesca rosea*. The pigment, a bright pink, follows the venation of the petals,—it is very pale at the edges and deepens toward the base, due to the convergence of the veins.

A plant homozygous for pink flowers and red fruit was crossed with one homozygous for white flowers and white fruit. Selfings and backcrosses of the F_1 plants thus obtained yielded data both on flower color and fruit

color. Since the record for both characters is not complete for every plant, the data for the two characters are recorded separately.

The F_1 plants were all pink-flowered, the shade being slightly paler than that of the pink-flowered parent. Four of the F_1 plants were selected for further study, each being selfed and backcrossed reciprocally to the recessive.

The totals obtained from selfing, 128 pink and 46 white, deviate only 2.5 from the numbers expected on a 3:1 basis, indicating the action of a single factor pair, which may be designated *Pp*.

When the heterozygotes *Pp* were used as females and backcrossed to the recessive male *pp*, 89 pink and 116 white were obtained, showing a deviation from expectation on a 1:1 basis of 13.5—almost three times the probable error, 4.8.

When the heterozygotes *Pp* were used as males and backcrossed to the recessive female *pp* a slight excess of white-flowered individuals was also obtained, 111 white to 93 pink. The deviation from expectation, however, is only 9, less than twice the probable error, 4.8.

RICHARDSON (1918) made a preliminary study of the inheritance of pink flower color without reaching a definite conclusion as to its inheritance, apparently because of difficulty in classifying the progeny. By selfing a plant heterozygous for pink flower color he obtained 20 pink, 57 pale pink and 10 white, or nearly white. It appears that he failed, in classifying his plants, to distinguish between a pinkish blush suffused throughout the entire petals—a condition very frequently found, especially in the newly opened flowers—and the definite pink venation pattern resulting from the action of the factor under observation.

Fruit color. The white fruited form used in these studies was obtained from the UNITED STATES DEPARTMENT of AGRICULTURE under the name *F. americana alba*. It is a typical *vesca* except for the color of the fruit, which is creamy white. The seeds of the red-fruited form are of a dark mahogany color, those of the white fruited form are straw colored.

Red fruit color is almost completely dominant over white. The fruits of the F_1 plants were only a trifle lighter in color than those of the red-fruited parent. As in the flower color crosses, the four plants studied include selfings and reciprocal backcrosses to the recessive.

When the four heterozygotes were selfed 102 red-fruited and 56 white-fruited plants were obtained. On the basis of a 3:1 segregation the expected numbers are 118.5 and 39.5, the whites showing a considerable excess (16.5, or 4.4 times the probable error) over expectation. The reason for this excess is unknown, since the fact that the inheritance of the charac-

ter is conditioned by the action of a single factor pair *Rr* is clearly shown by the backcross data.

When the heterozygotes *Rr* were used as females in backcrosses to the recessive male *rr*, 88 red-fruited and 91 white-fruited plants were obtained,—a very close approximation to the expected 1:1 ratio.

In the reciprocal of this cross, 84 red-fruited and 97 white-fruited plants were obtained. The expectation on a 1:1 basis is 90.5, the deviation 6.5 and the probable error 3.8.

RICHARDSON (1914) obtained in the selfed progeny of a plant heterozygous for white fruit 70 red-fruited and 20 white-fruited plants—a very close approximation to a 3:1 ratio.

In Table 1 the relation of flower color and fruit color to each other is considered. The heterozygotes *Pp Rr* were here used as females with the double recessive *pp rr*. The parental classes, that is those with pink flowers and red fruit, show a total of 89, as against 90 in the recombination classes, a clear case of free recombination.

TABLE 1

Test for linkage between red fruit color and white flower color, heterozygote PpRr×double recessive.

FAMILY	PARENTAGE	*PR*	*Pr*	*pR*	*pr*
202	107-2×Double recessive	4	7	11	13
205	107-4× " "	15	21	21	23
208	107-6× " "	5	1	6	4
211	108-1× " "	14	11	12	11
Total		38	40	50	51

Total for parental classes	89
Total for recombination classes	90
Expected on basis of free recombination	89.5
Deviation	.5
Probable Error	4.5
Dev.÷P. E.	.1

Finally in table 2 are listed the results obtained when the heterozygotes *Pp Rr* are used as males in backcrosses to the double recessive *pp rr*.

The development of seeds without true fertilization, by means of apogamy or parthenogenesis, might be expected to result, in this cross, in an excess of individuals like the female parent, that is with white flowers and fruits. A slight excess was, in fact, obtained, there being 57 of this class as against 45 expected. The deviation from expectation is 12, the probable error 4. The figures suggest that parthenogenesis or apogamy

may have been present, but the excess is hardly great enough to be significant. It must be remembered, also, that a similar excess could result from accidental selfing.

TABLE 2

Test for linkage between red fruit color and white flower color, double recessive×heterozygote PpRr.

FAMILY	PARENTAGE	*PR*	*Pr*	*pR*	*pr*
201	Double recessive×107-2	5	8	10	7
204	" " ×107-4	8	6	4	6
207	" " ×107-6	11	11	16	24
210	" " ×108-1	13	16	17	19
Total		37	41	47	56

Total for parental classes	93
Total for recombination classes	88
Expected on basis of free recombination	90.5
Deviation	2.5
Probable Error	4.5
Dev.÷P. E.	.6

A test for the presence of pseudogamy, that is, the parthenogenetic or apogamic development of seed brought about through the stimulus of pollen tube penetration, may be made by pollinating a recessive form under the most carefully guarded conditions with pollen from a homozygous dominant. Even under these circumstances the appearance of recessives in the progeny cannot be regarded as positive proof of pseudogamy, since mutation in the pollen to the recessive factor would produce a similar result. If, however, two factors instead of one are followed, this difficulty is eliminated, the likelihood of a simultaneous mutation in both factors being too remote to be considered. The most serious danger of error in such a test is, of course, that of accidental selfing. The significance of the results obtained must depend largely upon how carefully this danger is guarded against.

A preliminary test carried out according to the plan just described has been made on a small scale. A homozygous recessive white-flowered, white-fruited individual was pollinated with pollen from a homozygous dominant pink-flowered, red-fruited individual. The resulting progeny (Family 215) contained 27 individuals all of which were pink-flowered and red-fruited. The fact that no recessives appeared, however, does not justify any positive statements as to the presence or absence of pseudogamy. Different results might be obtained under different environmental conditions. Furthermore, the number of plants under observation is

too small to be very significant. It is also quite possible that parthenogenesis or apogamy, if they occur in Fragaria as indicated by our other crosses, result only when true fertilization is rendered difficult by the use of pollen from more distant species.

MILLARDET'S "FAUX HYBRIDES"

Few cases of anomalous inheritance are cited more frequently than the Fragaria hybrids of MILLARDET. In fact, it was the exceptional nature of his results that led to our re-examination of the behavior of species hybrids in this genus.

MILLARDET'S investigations, carried on at Bordeaux, where he occupied the botanical chair on the Faculté des Sciences, were begun in 1883 and extended over a period of eleven years. The thesis to which his observations led him is concisely stated in the introduction to his paper in the following words:

"Dans son important ouvrage sur l'hybridation des végétaux, Gärtner formule cette loi que 'nous ne connaissons aucun cas où le type d'une des espèces composantes d'un hybride ait passé intégralement dans ce dernier'. Tous les physiologistes qui se sont occupés d'hybridation, avant comme après lui, ont partagé cette même opinion. Mon intention, dans ce travail, est de démontrer que dans le genre Fraisier (*Fragaria*) les produits obtenus par l'hybridation de certaines espèces font exception à la règle générale qu'on vient de lire. Ils reproduisent intégralement le type spécifique du père ou celui de la mère, et ressemblent par conséquent exclusivement soit à l'un, soit à l'autre, sans réunir jamais à la fois aucun des caractères distinctifs des deux espéces composantes."

The results of the crosses upon which he bases his conclusions will be discussed in the order in which he presents them.

The first cross described is one in which "Fraisier des quatre saisons blanc" is used as the female parent and "Chili velu" is used as the male parent. The female parent, according to his description, is *F. vesca*, variety *alba;* the male parent is an hermaphroditic variety of *F. chiloensis* He obtained 4 hybrids, of which 1 resembled the female parent in every respect except that the fruit was *red;* the others resembled the male parent "from which it was almost impossible to distinguish them."

The *vesca* group, to which the female parent belongs, has 7 pairs of chromosomes; *F. chiloensis*, the male parent, has 28 pairs. In our own progenies from crosses between these two groups there have appeared individuals which displayed a marked dominance in F_1 of the 28-chromosome type over the 7-chromosome type. The dominance is not complete for every character, certain features such as flower form, for example, being

intermediate between the two parents; but that MILLARDET should have seen in these three plants a "reproduction du type specifique paternel" is not surprising.

Even in the absence of other evidence, it is fairly safe to conclude from the partial sterility of these three plants that they are true hybrids.

> "Les fleurs en sont grandes, hermaphrodites, mais a étamines assez peu dévelopées. Les anthères sont à peu près normales ainsi que le pollen. . . . . Toutes trois sont fertiles, mais médiocrement."

Our own hybrids between these two groups have thus far proved to be completely sterile. RICHARDSON (1918), however, obtained a slight degree of fertility in hybrids between *F. vesca* and *F. virginiana*, a cross involving the same chromosome numbers as the one under discussion.

The progenies of these 3 plants gave further evidence of the hybrid sterility which is to be expected in crosses between the 7-chromosome and the 28-chromosome group. Thus sowing "*R*" produced 2 plants "du type *chiloensis* pur. Ces plantes ont toujours été infertiles. Étamines et anthères peu développées. Pollen imparfait ou nul." MILLARDET stresses the fact that the plants are "paternal" in type, their sterility he evidently considers incidental and of no particular significance.

Several other sowings were made from these 3 plants, mostly from open pollinated seed. MILLARDET emphasizes the fact that in none of these progenies was there to be found the "slightest trace" of *vesca* characters. No conclusions can be drawn from these progenies since the pollen parents are unknown. If the pollen were supplied by one or more species of the 28-chromosome group, the *vesca* characters would again be covered up.

Let us return to the first of the 4 hybrids which MILLARDET describes as being "exactement semblable a la mère, sauf les fruits, qui sont rouges." The fact that this plant was red-fruited, while its mother was white-fruited does not trouble MILLARDET. In speaking of this family in another place, he says

> "dans l'exemple 1, sur quatre hybrides, tandis que trois reproduisaient le type du père, un reproduisait celui de la mère, avec cette variante *sans importance* que les fruits au lieu d'être blancs étaient rouges." (The italics are our own).

It will be recalled that fruit color in *F. vesca* depends upon the interaction of a single pair of factors, white being recessive to red. Red-fruited individuals have the constitution *RR* or *Rr*, white-fruited, *rr*. The appearance of a white-fruited individual in the progeny of a red-

fruited plant might be explained without much difficulty since the mother could have been heterozygous for the *R* factor; in such a case white-fruited offspring resulting from the parthenogenetic development of reduced eggs would have been at least a theoretical possibility. Even mutation as an explanation might have some plausibility if the change had been from red to white, for loss of color is rather common in plants; but there are few cases in the literature of white mutating to color. Apparently the reaction is not easily reversible.

Assuming that the plant is really of the parentage which MILLARDET assigns to it, only one explanation suggests itself for the red-fruit color (except the improbable one of mutation). Had it received the full complement of 28 chromosomes from its male parent, *F. chiloensis*, it would have shown the dominance of *chiloensis* characters, as did the other three members of the progeny. ICHIJIMA (1926) has found that at the reduction division of the hybrids between the 7-chromosome and 28-chromosome groups, 7 bivalents and 21 uni valents can be distinguished. It appears that the 7 *vesca* chromosomes pair with 7 of the 28 chromosomes from the other parent, leaving 21 unpaired. It is conceivable that at the first division following the fusion of the male and female nuclei the unpaired chromosomes might be left behind, resulting in an individual with 7 pairs of chromosomes, like the female parent in appearance but having red fruit. That a plant receiving such a complement of chromosomes would appear *vesca*-like is purely an assumption, nor is there any evidence on the question as to whether the unpaired chromosomes might be left behind at the first division of the newly formed zygote, as postulated.

There is, however, a bit of evidence which might lend credence to the possibility. The F_1 individuals resulting from species crosses of this type are extremely variable, ranging from vigorous plants entirely normal in appearance except for their sterility, to tiny dwarfs which can be kept alive only with difficulty. Varying degrees of chromosome elimination might conceivably bring about such results as those which MILLARDET obtained. But since no case has been found agreeing in every detail with that of the plant under discussion, the likelihood that the explanation suggested is the correct one appears to be very remote.

It seems much more probable that this red-fruited *vesca* plant is the result of one of several possible forms of contamination. The seed may have been present in the soil in which the sowing was made, *F. vesca* with the red fruits being the common wild strawberry of France. MILLARDET states that all sowings were made in "terre de bruyère" procured directly

from the forest. This practice may have reduced the danger of contamination from foreign seed, but it certainly cannot have removed it entirely.

Another possible source of error comes from trespassing runners. Unless the plants are kept constantly under observation a runner from a neighboring plant may, in a very short time, become established on the spot left vacant by the death of the original plant, and once the connection of the intruder with its parent is broken, as usually happens when the bed is cultivated, the evidence as to its origin is destroyed. A third and most probable source of contamination is that of wind-borne pollen. MILLARDET recognized the possibility of contamination from this source, but apparently he did not regard it as serious. He describes the cages used to protect the plants as being

"garnies de tulle, et quelquefois même de mousseline très fine, de manière à empêcher complètement non seulement l'accès des insectes, mais aussi, lorsqu'elles étaient couvertes de mousseline, la pénétration du pollen étranger qui aurait pu être apporté par le vent."

Certainly the cages which were covered with tulle offered practically no protection, and it is questionable whether those covered with muslin would exclude entirely pollen grains as small as those of Fragaria.

Most of the remaining crosses reported by MILLARDET present difficulties of interpretation similar to those just discussed. It is hardly desirable to consider each cross in detail. The results which he obtained will be briefly set down, however, as a matter of record.

"*Hybridation No. 10.*" Black Hautbois (*F. elatior*—21-chromosome group—the parenthesized explanatory notes being our own) × Globe (cultivated variety—28-chromosome group). Fifteen plants were grown from the cross; 14 were exactly like the female parent, 1 was exactly like the male parent.

"*Hybridation No. 8.*" Fraisier des quatre saison blanc (*F. vesca*—7-chromosome group) × Globe (cultivated variety—28-chromosome group). Only 1 plant resulted from this cross. MILLARDET describes it thus:

"La plante est vigoureuse et fleurit abondamment. Les fleurs sont petites (de la grandeur des fleurs femelles de *Virginiana* et *Chiloensis*), à étamines atrophiées et à anthères de couleur sale, ne contenant pas de pollen. Comme la plante s'est toujours montrée stérile, on doit en inférer que ses ovules ne sont pas en meilleur état que les anthères. Dans cet exemple, l'hybridation a reproduit le type spécifique paternel sans aucun mélange des caractères du type maternel, mais il y a impuissance complète tant des organes femelles que des mâles."

The plant is obviously a true hybrid with the characters of the 28-chromosome species dominant, as is usually the case in crosses between these two groups.

"*Hybridation No. 16.*" Fraisier des Alpes (*F. vesca*—7 chromosome group)×Ananas (cultivated variety—28-chromosome group). This cross yielded 24 plants, of which all but 1 were exactly like the female parent. The exceptional plant

> "reproduit le type paternel sans mélange de caractères étrangers à ce type. Mais cette plante est un peu rabougrie; elle fleurit peu et n'a jamais porté de fruits. Les fleurs sont petites, à étamines et anthères atrophiées, tout à fait semblables aux fleurs de la plante dont il vient d'être question (hybride 8)."

This plant, like the one in the preceding cross, is undoubtedly a true hybrid with dominance of the paternal characters.

"*Hybridation No. 1.*" Belle Bordelaise (*F. elatior*—21-chromosome group)×Gaillon rouge (*F. vesca*—7-chromosome group). The family consisted of 3 plants, all like the female parent. They were fertile, and therefore must have resulted either from induced parthenogenesis or apogamy, or from contamination.

"*Hybridation No. 2.*" Black Hautbois (*F. elatior*—21-chromosome group)×Docteur Nicaise (cultivated variety—28-chromosome group). This family consisted of 8 plants, all like the female parent. Again there is pseudogamy or contamination.

"*Hybridation No. 3.*" Black Hautbois (*F. elatior*—21-chromosome group)×Chili velu (*F. chiloensis*—28-chromosome group). The family consisted of 8 plants, all like the female parent. Again there is pseudogamy or contamination.

"*Hybridation No. 4.*" Black Hautbois (*F. elatior*—21-chromosome group)×Gaillon Rouge (*F. vesca*—7-chromosome group). The family consisted of 10 plants, all like the female parent, and which, therefore, must have arisen through pseudogamy or through contamination. Our own crosses between the 7-chromosome group and the 21-chromosome group gave hybrids which never developed beyond the seedling stage.

Open pollinated seed from these hybrids gave, besides maternals, 2 plants of the 28-chromosome type, which presumably was due to the dominance of the characters transmitted through pollen from plants of that type. In addition 1 plant showing pure *vesca* characters appeared. Contamination in the seed pan or the trespassing of runners is the only explanation that can be offered for this plant. CORRENS (1901) comments on it thus:

"Ich weiss erst seit ein paar Wochen privatim, durch die Liebenswürdigkeit MILLARDET's, dass sich hier ein Irrthum eingeschlichen hat. . . . . Es waren mit der zur Cultur verwandten Haideerde Samen der wilden *Fragaria vesca* eingeschleppt worden. Herr Professor MILLARDET hat mir gütigst erlaubt, dies zu veröffentlichen."

"*Hybridation No. 13.*" Black Hautbois (*F. elatior*—21-chromosome group)×Docteur Nicaise (cultivated variety—28-chromosome group). 3 plants, all maternal.

"*Hybridation No. 14.*" *F. elatior* (21-chromosome group)×Ananas (cultivated variety—28-chromosome group). 9 plants, all maternal.

"*Hybridation No. 17.*" *F. elatior* (21-chromosome group)×*F. virginiana* (28-chromosome group). 9 plants, all maternal.

"*Hybridation No. 20.*" *F. vesca* (7-chromosome group)×*F. elatior* (21-chromosome group). 5 plants, all maternal.

"*Hybridation No. 23.*" Fraisiers des Alpes (*F. vesca*—7-chromosome group)×*F. elatior* (21-chromosome group). 3 plants, all maternal.

"*Hybridation No. 25.*" Hybride 11–3×*F. elatior* (21-chromosome group). 2 plants, all maternal.

"*Hybridation No. 35.*" Docteur Nicaise (cultivated variety—28-chromosome group)×*F. vesca* (7-chromosome group). 4 plants, all maternal.

"*Hybridation No. 39.*" *F. vesca* (7-chromosome group)×*F. virginiana* (28-chromosome group). 1 plant, maternal.

"*Hybridation No. 45.*" *F. californica* (7-chromosome group)×Docteur Nicaise (cultivated variety—28-chromosome group). 9 plants, all maternal.

"*Hybridation No. 46.*" *F. vesca* (7-chromosome group)×*F. californica* (7-chromosome group). 6 plants, all maternal.

One may sum up MILLARDET'S results as follows: He made many crosses between different species of Fragaria using methods of control which are satisfactory for most plants, but which we have found unsatisfactory for Fragaria where the pollen is extremely small. He dealt with general resemblances in species which differ from each other by rather vague quantitative characters, and was not impressed with minor, individual characters, with dwarfness, or with sterility, as genetic evidence. Many of his populations came from open-pollinated plants. Some of the aberrant plants which appeared, therefore, almost certainly resulted from pollen, seed, or runner contamination.

In addition, there are two phenomena to be explained. In the first place, many plants appeared which were of the type of the female parent.

These maternal plants may have resulted either from accidental pollination with pollen of the same general type, or from pseudogamy. We are not prepared at present to accept either alternative to the exclusion of the other, but it cannot be denied that on general grounds pseudogamy is a possibility. In addition, there are four cases where individuals were obtained which resembled the male parent, excluding two instances of open pollination where the male parent was unknown. In two of these cases, *F. vesca*×*Globe* and *F. vesca*×*Ananas* (7-chromosome type×28-chromosome type), the exceptional individuals, two in number, showed their hybrid nature by being completely sterile. The other two cases were *F. vesca*×*F. chiloensis* (7-chromosome type×28-chromosome type) and *F. elatior*×*Globe* (21-chromosome type×28-chromosome type). In each instance these plants showed their hybrid nature by greatly reduced fertility. They did indeed yield a few F_2 progeny from open-pollinated seed, which were somewhat variable, but which in general resembled the plants of the F_1 generation. These results, therefore, show only the dominance of the type with the higher chromosome number which is usually to be expected when plants are crossed which have markedly different chromosome numbers, and the unequal distribution of characters which naturally occurs under such circumstances. On the other hand, the case where the 28-chromosome type dominates the 21-chromosome type is in direct opposition to our own results. In our experience, the characters of the 21-chromosome type dominate the characters of the 28-chromosome type as well as the characters of the 7-chromosome type; but this is contrary to the experience of investigators on other genera with crosses showing analogous chromosome differences.

The results obtained by SOLMS-LAUBACH (1907) have been cited more than once as confirming the conclusions of MILLARDET. Indeed, it appears that SOLMS-LAUBACH himself believed that such was the case. He speaks of his findings as being "in schönster Uebereinstimmung mit den Ergebnissen jenes Autors."

SOLMS-LAUBACH made several crosses between a pistillate form of *F. virginiana* as the female parent and *F. elatior* as the male parent. The heavy pubescence, the shape of the leaves, their light, slightly greyish green color, their deeply impressed venation and the general growth habit of *F. elatior* have proved in our own crosses to be almost completely dominant over the corresponding characters in *F. virginiana*. SOLMS-LAUBACH obtained a similar result, his experience being therefore different from that of MILLARDET.

"Aus den Samen der *F. virginiana* war also hier fast ganz reine *elatior* (moschata) auf gegangen, es war 'fécondation sans croisement' in schonster Form eingetreten."

The plants which result from crosses between these two species are completely or almost completely sterile; SOLMS-LAUBACH observed the fact but appears to have attached no significance to it.

It is obvious that here again we are dealing with true hybrids showing a dominance of the paternal type and displaying the sterility characteristic of hybrids between species differing in their chromosome numbers.

GIARD (1903) interprets the patroclinous hybrids of MILLARDET as having a basis similar to that of the development of spermatozoa in enucleated sea urchin eggs as observed by BOVERI (1895) and DELAGE (1899). This hypothesis is almost certainly incorrect. ICHIJIMA (loc.cit.) has made chromosome counts on several of such patroclinous hybrids and finds that they contain the sum of the haploid numbers of the parental species, indicating that the nuclei of both hybrids participated in the formation of the zygotic numbers.

The matroclinous plants which constituted a large part of most of MILLARDET'S progenies GIARD believes have resulted from a parthenogenetic development of the egg, induced by stimulus from the foreign pollen tube. They may have originated in this way, but it must be kept in mind that they may also have resulted from accidental selfing. In our own crosses between different species, diploid individuals of the maternal type have appeared from time to time. Furthermore, apogamy is a regular occurrence in other Rosaceæ, notably in *Rosa* (TACKHOLM, 1922 and LIDFORRS, 1914). It seems significant, however, that with the adoption of more rigorous measures to prevent contamination, such individuals have appeared less and less frequently in our cultures.

The most recent reference to the work of MILLARDET is that of LONGLEY (1926). He describes the results of a cytological study of various Fragaria forms, among them one matroclinous and one patroclinous hybrid, which he apparently believes afford further evidence in favor of MILLARDET'S assumption that a unique form of inheritance obtains in Fragaria.

The matroclinous plant appeared in the progeny of a cross between *F. vesca* (7-chromosome group) and *F. chiloensis* (28-chromosome group). This plant was found by LONGLEY to contain 7 bivalent chromosomes—the same number as in the female parent. This observation, however, throws no light on the question as to whether such plants have resulted from parthenogenesis or from accidental selfing.

The second plant appeared in the progeny of a cross between *F. vesca* var. *alba* (7-chromosome group) and the cultivated variety Aroma (28-chromosome group). It resembles the male parent morphologically, and was found by LONGLEY to have the same chromosome number. Neither of these facts is particularly significant in establishing whether the plant is actually the product of the cross from which it is supposed to have originated. Here again the question arises as to whether the sowing was made in sterile soil, and whether the plant was kept constantly under observation from the seedling stage until maturity. Nevertheless, the possibility that the chromosomes of the female may somehow be lost at the fusion of the two gametes, resulting in an individual which duplicates the male parent, is certainly not to be excluded. The Tripsacum-Euchlaena hybrid of COLLINS and KEMPTON (1916) which, both in appearance and in three generations of breeding, showed no trace of any of the characters of the female, appears to furnish an example of some such phenomenon.

CONCLUSIONS

Fragaria species having the same number of chromosomes cross freely, yielding perfectly fertile hybrids in which segregation takes place in the normal manner. Typical segregation in sex manifestations was observed in crosses between 28-chromosome species, while normal segregation of two independent pairs of allelomorphs, pink flowers and white flowers and red fruit and white fruit, was observed on fairly large populations in crosses between 7-chromosome species.

Crosses in which *F. elatior*, the 21-chromosome species, was used as the female and 7-chromosome species as the male, resulted in failure. In the reciprocal crosses, fruit set readily; but the seeds showed only about 0.7 percent germination, and the few seedlings obtained died within two weeks.

Crosses in which the various 28-chromosome species were used as the female and the various 7-chromosome species as the male also failed; but reciprocal crosses were made without difficulty. Many of the achenes were poorly developed and germination was low. Three types of plants appeared in the F_1 generation; vigorous hybrids showing a more or less complete dominance of the 28-chromosome type, dwarfs which did not flower, and maternals. The hybrids were completely sterile.

A pistillate individual of the 28-chromosome group set fruit readily with pollen from *F. elatior* (21-chromosomes). The seeds germinated well (90 percent). The plants were variable in vigor, but showed a striking

dominance of the *F. elatior* characters. They were completely sterile. Maternals were also obtained.

The flowers of 7-chromosome species set fruit readily with pollen from *Duchesnea indica* (42 chromosomes). From 500 seeds, 30 plants were obtained. Of these 24 were small, weak hybrids; the remainder were maternals.

Two seedlings were also obtained from *F. vesca* (7 chromosomes) flowers fertilized with the pollen of *Potentilla nepalensis*.

Though rare cases of parthenocarpy were observed, pseudogamy was not induced by treatments given, unless the maternal individuals appearing in the crosses between species differing in chromosome number are such. These maternals have the diploid number of chromosomes characteristic of the species involved, and may result either from induced parthenogenesis (haploid development) with a doubling of the chromosomes during early development, or from induced apogamy (diploid development). But since the pollen in all the Fragaria species is extremely small and easily carried by wind currents, we are not prepared to maintain that these plants did not result from accidental selfing.

MILLARDET's plants of maternal type must have been, like our own, the result either of pseudogamy or of selfing. His plants of paternal type were all obtained by crossing species with a lower chromosome number with species with a higher chromosome number, and were, we believe, merely hybrids showing general dominance of the type with the higher chromosome number. There is no evidence, therefore, that inheritance in Fragaria is of a new or unusual kind as has been so often maintained.

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THE FUNCTIONAL INTER-RELATION OF CERTAIN GENES IN THE DEVELOPMENT OF THE MOUSE

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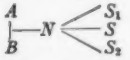
INTRODUCTION

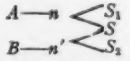
While it is obvious that somatic traits are largely determined by genes in the chromosomes, the precise way in which the genes produce their effect is as yet unknown. Two possibilities suggest themselves. Genes located in the same nucleus may mutually react with each other in such a way as to give a distinctive resultant effect which will produce characteristic somatic manifestations, or each gene may severally and independently exert its own peculiar influence upon the surrounding cytoplasm.

In the first case the relation between genes and soma might be indicated as



in which A and B represent genes, N the cytoplasm of a cell, and S, S_1, or S_2, its possible contributions to the soma. Whether the particular contribution of the cell shall be S, S_1, S_2 $\cdots$ will depend on its topographical position and such other factors as are studied by embryologists and experimental morphologists, but the essential character of the nuclear influence will be fixed throughout by the nature of the interaction between A and B.

The second possibility may be indicated as



in which n and n' represent independent reactions of A and B with the cytoplasm and stand for two units in the sum total of cytoplasmic potentialities. In this case A and B might both contribute to the deter-

mination of S, while B would have no part in the determination of S_1, and A none with reference to S_2. According to the first supposition if B modifies the effect of A on one trait it should also modify its effect on any other trait, even with full allowance for cytoplasmic differentiation and the action of environmental factors; while according to the second, B might influence the effect of A on some traits but not on others.

A figurative statement of the problem is suggested by an analogy employed by CONKLIN (1920, p. 130) in which it is suggested that the genes "represent the differential causes of particular characters just as in the compounds H_2SO_4 and K_2SO_4 the hydrogen and potassium atoms represent the differential causes of the properties manifested by these two substances." Continuing the analogy essentially in this form, one might picture the nuclei of one idividual as "H_2SO_4" in their reaction with the cytoplasm at successive developmental stages, while the nuclei of another individual would react as "K_2SO_4." This accords with the first alternative, where the difference is not simply between "H_2" and "K_2", but between the compounds "H_2SO_4" and "K_2SO_4." On the second assumption the analogy of a chemical compound breaks down. In place of the compounds are the elements "H" or "K", "S," and "O," each tending to form its own independent reactions with the cytoplasm. Either arrangement would seem to provide the possibility of a great many "unit characters," but the implications of the two hypotheses are quite different.

An approach to the solution of this problem is afforded by cases in which the same primary gene produces two or more distinct effects, in connection with at least one of which some other known gene is also operative. A^y and W in the mouse and the genes which affect their expression, present situations of this sort.

THE A^y GENE IN MICE

The same gene that determines yellow coat color also results in an increased adiposity after maturity. (DANFORTH, 1926). These traits occur when the gene is in the heterozygous condition. Individuals homozygous for it are apparently unable to survive the early embryonic period (KIRKHAM 1917, IBSEN and STEIGLEDER 1917), so this gene may also be regarded as a lethal. Thus three very different effects of the A^y gene may be recognized: early death for homozygous individuals, yellow color and adiposity for the surviving heterozygotes. But as regards color, it is well known that a number of other genes are effective in modifying or suppressing yellow in the pelage. Among these are the genes for dominant (Ww) and recessive (ss) spotting and that for albinism (cc).

Now, since a mouse with the genetic formula *CCSSA^{v}a* has a yellow pelage and becomes obese, what will be the character of a mouse with some modification of this formula such as *CCssA^{v}a* which has only a limited amount of yellow, or *ccSSA^{v}a* which has no yellow at all? If the genes *s* and *A^{v}*, and *c* and *A^{v}* interact directly with each other and indirectly with the cytoplasm it might be expected that the spotted mouse would show only a limited tendency to adiposity and the albino none at all; but if they do not interact directly the effect of these genes on adiposity could not be predicted from their effect on color.

Strains with various formulae have been made up and tested for possible relations between color and adiposity. Table 1 is an abstract of a series of data on the weights of three pairs of litter-mate sisters. The two members of each pair were kept in the same cage, supplied with the same

TABLE 1

Weights of mice of different genotypes with reference to the presence or absence of A^{v}.

GENOTYPE	COLOR	WEIGHT IN GRAMS AT SUCCESSIVE AGES			
		1 day	3 months	6 months	15 months
CCS-A^{v}a	Yellow	1.60	30	42	56
CCS-aa	Black	1.78	21	23	32
CCssA^{v}a	Spotted Yellow	1.46	33	52	69
CCssaa	Spotted Black	1.60	23	34	52
ccSSA^{v}a	White	..	25	48	64
ccSSaa	White	..	22	25	30
Averages	*A^{v}*	1.53	29	47	63
	a	1.69	22	27	38

food, and in every respect given as nearly identical treatment as possible. In these three sets the *A^{v}* gene is brought into relation with a combination which permits a full expression of its effect on color, one which restricts its expression, and one which wholly suppresses it. In each case, however, the individual carrying the *A^{v}* gene becomes much heavier than the corresponding control which lacks it. Similar results have been obtained in other controlled experiments, and have been confirmed no less convincingly by the repeated occurrence of typical adiposity in dominant spotted, recessive spotted, "sooty" and albino individuals which were known to carry *A^{v}*, but which had not been especially bred to test the point in question. These observations make it clear that the ability of the *A^{v}* gene to produce adiposity is not related to its opportunity to affect color.

The other well known action of the A^y gene is its lethal effect when tomozygous. Since no homozygous strain of ordinary yellow, sable, hnk-eyed, or spotted yellow has ever been obtained, the combined experience of many investigators may be taken as evidence that the genes which thus limit or modify the effect of A^y do not prevent the lethal action of A^yA^y. It remains, however to determine whether or not albinism which causes a complete failure of the A^y gene as a color factor also suppresses it as a lethal. This point was tested by matings between individuals with the formula ccA^ya, which should produce some zygotes of the constitution ccA^yA^y. The stock was obtained from an original mating of an albino female and a yellow male. The first generation consisted of some yellow and some black offspring. The latter were discarded and the yellows mated among themselves, giving in F_2 some albino and some colored young. The albinos were saved and tested by mating to non-yellow colored individuals. Those that produced any yellow young were shown by that fact to be of the formula ccA^ya. These were now mated together and their offspring, F_3, raised to maturity and tested as to their genetic constitution.

In young from the mating $ccA^ya \times ccA^ya$ the effect of A^y on color is wholly suppressed. If its lethal effect were also suppressed, one-fourth of the young should have the formula ccA^yA^y and produce only yellow young when bred to *CCaa* mates. The result of the tests is shown in table 2. Forty-four mice from $ccA^ya \times ccA^ya$ parents were found to include no ccA^yA^y individual. If ccA^yA^y were not lethal there should have been about eleven of this formula. Of these forty-four, 26 were found to be A^ya and 18 recorded as *aa*. Some of the latter, however, were possibly A^ya since each individual was classed as not A^yA^y and discarded as soon as it produced a non-yellow colored young. Thus seven mice were classified as not A^yA^y and probably *aa* on the basis of litters of five or less young. This may account for the slight deviation from the ratio of 29:15 which might have been expected on the assumption that A^yA^y is really lethal. If ccA^yA^y were not lethal, the chances against this combination occurring at least once among 44 mice of this ancestry are $(3/4)^{44}$, or among 26 yellow producers $(2/3)^{26}$. The odds against the failure of a ccA^yA^y individual to occur being a matter of chance are thus more than 30,000:1.

The data on 36 mice among which two or more *ccaa* individuals were inadvertently included are also given in table 2. This shows a total of 80 mice, the majority of which were suitable for critical tests, and all of which were shown by the 765 young which they produced not to be ccA^yA^y. It is consequently apparent that the combination *cc* which

suppresses the effect of A^v on color does not prevent it from being a lethal when homozygous.

TABLE 2

The distribution of young from $ccA^va \times ccA^va$ matings.

PARENTS	CONSTITUTION AS INDICATED BY CHARACTER OF OFFSPRING						
	ccA^va			*ccaa*			TOTAL
	Males	Females	Total	Males	Females	Total	BOTH TYPES
All *ccA^va*	18	8	26	6	12	18	44
Mostly* *ccA^va*	9	11	20	9	7	16	36
Total	27	19	46	15	19	34	80

* Possibly two or more *ccaa* individuals were included among the 36 in this line.

THE *W* GENE IN MICE

Somewhat similar tests were made with mice carrying the gene (*W*) for dominant spotting. When heterozygous this gene gives rise to a certain amount of white in the pelage; when homozygous, it produces a severe anaemia which terminates fatally within a few days of birth. (DE ABERLE 1925.)

The gene *W* may be introduced into a number of combinations, some of which have been discussed by DETLEFSEN (1916) and others by LITTLE (1917). The combinations *CCPPSSA^vaWw*, *CCPPSSaaWw*,

TABLE 3

The production of anaemia by mice in which different genes were associated with W.

GENOTYPE OF THE PARENTS	NORMAL YOUNG	ANAEMIC YOUNG	PERCENTAGE OF ANAEMICS*
C-P-ssaaWw (Black-eyed whites)	179	28	13.5
CCPPSSaaWw (Roans)	147	20	11.9
ccPPS—aWw (Albinos)	161	29	15.2
Totals	487	77	13.6

* The numbers in this column fall short of the expected 25 percent because some of the anaemics are born dead and others are lost between the inspections which in each case occurred at 24-hour intervals.

CCPPssA^vaWw, *CCPPssaaWw*, *CCppssaaWw*, and *ccPPSSA^vaWw* give a graded series ranging from animals that are almost completely pigmented to those with no pigment at all. In each of the complexes except the last, *W* is a factor in the production of the characteristic grade. Or, considering *W* as the constant, its effect on color is modified in one way

or another by each of these combinations. The *W* gene has been tested in each of the six combinations just mentioned (except that some of the individuals used were heterozygous for *C*, *P* or *S*). But none of the tests showed any marked effect on the incidence of anaemia (table 3). The relatively low frequency of anaemia in all tests is due to the poor viability of the anaemics and the fact that the cages were inspected only at 24 hour intervals. All classes produced anaemics in about the same proportion, and each of them produced perfectly typical representatives, those from the two extremes of the series, albinos and yellows, being quite as similar as those from any other two groups. This makes it certain that the genes which influence *W* with respect to color do not influence *WW* in any comparable way in respect to anaemia.

DISCUSSION

Since genes which modify or suppress the effect of A^y on color show no corresponding effect on its lethal action or its tendency to produce adiposity, and those which similarly influence *W* with respect to color do not affect the anaemia, it would seem that the second of the two suggested alternatives is the more nearly correct; that is, that genes which modify each other's effects do so indirectly and not through direct mutual interaction. If this conclusion is correct, and of general applicability, a commonly expressed view as to the interaction of genes is not tenable. In this case it is not *strictly* true that development consists in the "orderly interaction of these substances (genes)—with each other, with the rest of the cell body, or cytoplasm; and with the oxygen, food, · · · ·." (JENNINGS, 1924). The "*with each other*" should be deleted. If the seeming implications of the data are incorrect, it must be that we have been unfortunate in selecting examples where traits produced by the interaction of *A–N* and *AB–N* are indistinguishable while $A–N_1$ and $AB–N_1$ are widely different. (*N* and N_1, representing the cytoplasm at two different stages of development). However improbable, this is not entirely impossible.

A point of interest in this connection is the behavior of multiple allelomorphs. In Drosophila according to MORGAN (1926, p. 307) changes in the same locus affect all the traits which were affected by the original gene. This would seem to be confirming evidence from an entirely different angle. Instead of keeping one gene constant and varying its modifiers, the general complex is kept constant and the particular gene is varied. Nevertheless, the data presented here do not indicate that the relation mentioned by MORGAN is a necessary one and indeed evidence, which is not as yet adequate, suggests that it may not hold with the *A*, A^y, A^w, *a* series of multiple allelomorphs in the mouse.

The uncertainty in the situation arises from the fact that it is not entirely clear whether or not each mutant gene actually contributes to all of the reactions of the original or if some of the observed effects are due to the failure of the mutant to act at all in certain capacities. It is not surprising, however, that allelomorphic genes are frequently observed to affect the same series of traits, for if in the development of a normal individual a certain gene plays a necessary rôle in several vital reactions any mutant gene which replaces it must be able to somehow function in each of them or else behave as a lethal. Failure of mutant genes to meet this requirement may account for most lethals, and the likelihood of a viable mutation is probably quite as dependent on the number of "secondary" reactions to which the gene must contribute as upon the importance of the "primary" trait. It would seem that, other things being equal, viable mutations should most frequently be due to changes in genes which participate in a minimum number of reactions during development, and that they should most often affect "superficial" characters. But as yet there seems to be no real information as to just how a mutant gene fits into the place of the original.

Two possible objections to the procedure in this paper will possibly suggest themselves. It may seem that the idea of modifying genes is employed in an unjustifiable sense, that spotting, for example, does not affect *yellow* but *color distribution* and yellow merely happens to be the color in question. But wholly apart from the question of whether or not there are modifying genes in any other sense, it may be recalled that in the presence of W, yellow shows a very different degree of extension than does black or brown (LITTLE, 1917). Moreover, the question at issue really is this: Given a certain genetic constitution, does a new gene introduced into the stock by breeding produce its effect by reacting directly with the other genes introduced into the zygote at the same time or by reacting indirectly through the cytoplasm later on? For throwing light on this question the data under consideration would seem to be pertinent.

A more serious objection is that the three assumed effects of A^v may in reality be produced by three distinct but closely linked genes, and likewise that W is associated with a linked gene which is responsible for anaemia. It would seem impossible to entirely disprove such a hypothesis, but the probability against its being correct is extremely low in view of the immense number of mice that have been raised without a single case of crossing over between these traits having been observed. If, however, it should subsequently be shown that the effects which geneticists now

attribute to two genes, A^y and W, are in reality due to five different genes, the data and discussion in this paper would have very little point.

CONCLUSIONS

Several genes which influence the effect of A^y on color do not influence its effect on adiposity, or its lethal action when homozygous.

Similarly, combinations which influence the effect of W on color have no affect on anaemia.

These facts indicate that there is no constant interaction between the genes themselves, but that their somatic effects are produced by independent reactions with some other substance.

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DIFFERENTIAL ACTION OF THE SUGARY GENE IN MAIZE ON TWO ALTERNATIVE CLASSES OF MALE GAMETOPHYTES[1]

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INTRODUCTION

It was observed in 1924 that maize plants homozygous for the sugary gene and segregating for waxy, that is, of the formula $s_u\ s_u\ W_x\ w_x$, gave, when self-pollinated, only 18.8 percent of waxy grains. Since non-sugary, waxy heterozygotes on being selfed give, on the average, about 24 percent waxy seeds, plans were made for testing out the relationship which appeared to exist between the sugary and waxy genes in modifying the waxy ratio. The breeding facts which have been gathered reveal a situation of particular interest not only in its bearing on the rôle of the gametophyte of angiosperms in Mendelian inheritance but also on the broader problem of the relationship between cytoplasm and nucleus in heredity and determination. The results may serve, moreover, to give some further insight into the kinetics of the waxy and the sugary genes.

The sugary factor (s_u) serves to differentiate the so-called sweet corns from other types of maize. The gene produces its most conspicuous effect in the endosperm which in sugary races is wrinkled in characteristic fashion and typically vitreous in appearance. The endosperm is smaller

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and lighter in weight than in non-sugary seeds due to differences in the nature and amount of the reserves. Sugary behaves regularly as a simple recessive to the non-sugary condition.

The waxy gene (w_x) likewise occasions an alteration in the character of the endosperm. Seeds of this class have a dull, opaque appearance and the texture of a hard wax; they are only slightly lighter than non-waxy seeds and do not differ in shape. In contrast to those of all other types of maize the endosperm of the waxy race gives a reddish-brown color with the iodine-potassium-iodide reagent. It has been shown that this peculiar color reaction is due to the presence of an unusual type of starch. This action of the waxy gene on the carbohydrate reserves is also exemplified in the pollen. In homozygous waxy races the starch in the pollen stains reddish-brown with iodine. In segregating individuals 50 percent of the pollen grains give this reaction; the remainder contain the ordinary blue-staining starch. The recessive waxy character is the result of a simple factor difference.

In inheritance the sugary and the waxy genes are independent of each other, their location on different chromosomes having been definitely established by EMERSON (1924).

MATERIALS

The original stock in which the influence of the sugary gene on the waxy ratio was first noted was represented in 1924 by a single sugary progeny, R11, consisting of 63 plants. The seed planted came from a single ear resulting from a cross of the type $s_u\ s_u\ W_x\ W_x \times S_u\ s_u\ W_x\ w_x$. Of the plants which were self-pollinated, 25 proved to be segregating for waxy and yielded 31 ears. These ears bore 12,264 grains of which 18.84 percent were waxy. The proportion of waxy seeds expected on the Mendelian basis is, of course, 25 percent and the observed ratio is in defect of this by 6.16 $\pm$0.26 percent. The deviation, being over 23 times its probable error, is unquestionably significant.

Three non-sugary plants of the constitution $S_u\ s_u\ W_x\ w_x$ in progeny R13, a sib of R11, were also self-pollinated in 1924. These three ears yielded 1245 grains, 24.82$\pm$0.68 percent of which were waxy. This is 5.98$\pm$0.73 percent more waxy seeds than given by the homozygous sugary plants of like breeding. Since the difference between the sugary and non-sugary groups is over 8 times its probable error it is quite certainly not due to chance. These findings indicated an important effect of the sugary gene on the waxy ratio and plans were made to pursue the problem further.

In the 1925 plantings provision was made for determining the effect of the sugary gene on the waxy ratio in a detailed way. Selfed ears were obtained on sib plants of the constitutions, $S_u\ S_u\ W_x\ w_x$, $s_u\ s_u\ W_x\ w_x$ and $S_u\ s_u\ W_x\ w_x$. The following reciprocal combinations between sibs were also made: $S_u\ s_u\ W_x\ w_x$ with $s_u\ s_u\ W_x\ w_x$ and $S_u\ S_u\ W_x\ w_x$ with $s_u\ s_u\ W_x\ w_x$. The plants used in these matings were all closely related, tracing back to two self-pollinated ears from R13 in 1924. These stocks are comprised in the progenies R21, R21a, R22 and R22a. Many of the tests made with these materials were duplicated with two other sets of plants of like composition with regard to the designated genes but of distinct breeding. The latter stocks are represented by the groups R23, R23a and R24, R24a, R24b, R24c, respectively. Their origin will be given below.

A number of crosses were made using pistillate plants of the types $S_u\ S_u\ w_x\ w_x$ and $s_u\ s_u\ w_x\ w_x$. These individuals were of diverse breeding except in the cases of progenies R27, R28, R28a, R28b and R36 which consisted of immediate descendants of self-pollinated plants in the sib progenies, R11 and R13.

The above material enables us to compare the waxy ratios in sugary and non-sugary stocks of like breeding with respect to other genetic factors. Furthermore, they provide for this comparison in three groups of distinct origin, making possible an estimate of the extent to which the relationship probably holds generally.

ACCURACY OF CLASSIFICATION

The principal source of error in the data presented below undoubtedly lies in the classification of the seeds in segregating ears. Sugary and non-sugary grains can be distinguished easily enough on the basis of their clear-cut difference in appearance and present no difficulties. Separation of waxy grains from non-waxy, particularly in the sugary class, is not so readily accomplished. In races of maize with uncolored, plump endosperms waxy seeds may often be distinguished by simple inspection. Immaturity, or color in the aleurone layer, frequently obliterates the outward difference, however. In such cases chipping the endosperm with a knife serves to differentiate the two types on the basis of texture; or the difference in staining reaction with iodine noted above may be resorted to. In classifying sugary seeds for the waxy and non-waxy characters the use of an iodine solution is essential. Usually some "soft starch" is present in the vicinity of the embryo. If this soft starch area is exposed by cutting and the reagent applied to it the color difference resulting serves as a certain

means of distinguishing the two classes. If the sugary seeds are very hard and translucent they must be split through the embryo before staining but it is usually possible by chipping near the base to classify the seed and retain it in a germinable condition. We find it helpful to use a stronger solution of iodine in separating waxy and non-waxy sugary seeds than is otherwise called for. A preparation found very satisfactory contains 1.0 gram KI and 0.6 grams I in 100 cc of water. Sugary seeds with pigment in the aleurone layer may readily be bleached by warming in a weak solution of sulfurous acid. If the grains are then washed with water and dried the iodine reaction may be readily obtained.

We have sought to measure the amount of error resulting in the classification of this sort of material. A group of 17 ears segregating for both sugary and waxy formed the basis of the study. The grains on each ear were separated into the four component classes by a first operator; each seed within the four groups was then checked independently by a second individual. When an apparently misclassified seed was found the matter was checked by examination under a microscope, if necessary, where the two types of starch grains, waxy and non-waxy, show a distinct difference in reaction to iodine. The second classification was used as the standard against which the errors in the first were measured. With regard to the waxy grains in the non-sugary group, no errors were found in 9 of the 17 ears. In the remaining 8 ears, 16 grains in all were misclassified. The total number of non-sugary seeds was 4097, which gives a percentage error equal to 0.39. However, 10 of the misclassified grains had been entered in the waxy class and 6 in the non-waxy group. These errors tend to cancel each other giving a net discrepancy negligible in size. In the sugary class the error is larger. Of the 17 ears, 7 were correctly classified. Among the sugary grains in the remaining 10 ears, 33 were incorrectly identified. The error here amounts to 0.71 percent of the total, 4663 seeds. In 28 of the 33 cases the frequency of the waxy class was too high, which rather definitely indicates a tendency for the errors to go in this direction. In the discussion below we shall be concerned with waxy ratios in the sugary class distinctly lower, in general, than those expected on the Mendelian basis. Assuming that the results obtained with this group of 17 ears are representative, the systematic error arising in classification tends to bring these ratios toward the "expected" value, then, rather than to exaggerate the difference. This being so, it would seem unnecessary to qualify our conclusions in regard to the significance of the departures from the theoretical ratios on account of possible errors in classification.

RATIOS OBTAINED ON SELFING WAXY HETEROZYGOTES, HOMOZYGOUS FOR THE SUGARY AND THE NON-SUGARY GENES, RESPECTIVELY

Progenies R21 and R22 derived from selfed ears on sib plants comprised individuals of the constitutions $S_u\ S_u\ W_x\ W_x$, $S_u\ S_u\ W_x\ w_x$, $S_u\ s_u\ W_x\ W_x$, and $S_u\ s_u\ W_x\ w_x$. R21a and R22a, grown from the sugary seeds on the same ears, consisted of the genotypes $s_u\ s_u\ W_x\ W_x$ and $s_u\ s_u\ W_x\ w_x$. Since the waxy character is expressed in the pollen—waxy grains staining yellowish to brownish-red with iodine in contrast with the bluish shade given by non-waxy pollen—it is possible to distinguish the two genotypes $W_x\ W_x$ and $W_x\ w_x$ in the field at flowering time. Taking advantage of this fact, all the individuals in these four progenies were systematically examined and marked according to their composition with respect to the waxy gene before breeding work was begun. Plants homozygous or heterozygous for the non-sugary factor, however, can only be separated at maturity on the basis of the character of the seeds produced. It was expected, on Mendelian grounds, that roughly twice as many waxy heterozygotes would be heterozygous for non-sugary as homozygous for it and this prediction was borne out; 13 ears of the former type were obtained and 7 of the latter. The results with progenies R21 and R22, on the one hand, and R21a and R22a, on the other, will be thrown together to facilitate consideration.

TABLE 1

Distribution of waxy and non-waxy seeds obtained on selfing plants of the type $S_u\ S_u\ W_x\ w_x$ in progenies R21 and R22.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R21-16	436	113	549	20.58 ± 1.24	-4.42
-11	454	149	603	24.71 ± 1.19	-0.29
R22-56	251	99	350	28.29 ± 1.56	$+3.29$
-49	286	96	382	25.13 ± 1.49	$+0.13$
-48	244	62	306	20.26 ± 1.67	-4.74
-36	330	102	432	23.61 ± 1.41	-1.39
-25	354	106	460	23.04 ± 1.36	-1.96
Totals	2355	727	3082	23.59 ± 0.53	-1.41

The numbers of waxy and non-waxy seeds obtained on selfing plants of the type $S_u\ S_u\ W_x\ w_x$ are listed in table 1. These are to be compared with the ratios in table 2 relating to sib plants of the constitution $s_u\ s_u\ W_x\ w_x$. The proportion of waxy seeds on the non-sugary ears is 23.59 ± 0.53 percent as opposed to 18.37 ± 0.46 percent, or 5.22 ± 0.70 percent fewer

TABLE 2

Distribution of waxy and non-waxy seeds obtained on selfing plants of the type $s_u\ s_u\ W_x\ w_x$ in progenies R21a and R22a.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R21a-15	482	113	595	18.99±1.20	−6.01
-14	357	99	456	21.71±1.37	−3.29
- 5	355	89	444	20.04±1.39	−4.96
- 4	258	80	338	23.67±1.59	−1.33
-23	388	83	471	17.62±1.35	−7.38
R22a- 9	260	76	336	22.62±1.59	−2.38
-11	357	47	404	11.63±1.45	−13.37
-14	298	45	343	13.12±1.58	−11.88
-14	274	43	317	13.56±1.64	−11.44
-20	325	80	405	19.75±1.45	−5.25
Totals	3354	755	4109	18.37±0.46	−6.63

on the sugary ears. The odds are many thousand to one that this difference is statistically significant.

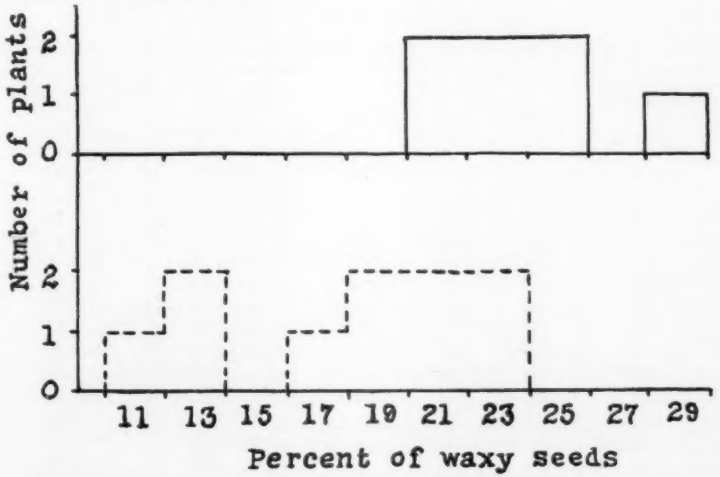

FIGURE 1.—Frequency distributions of plants, with respect to percent of waxy seeds produced on selfing in the non-sugary progenies R21, R22 (solid line) and the sugary progenies R21a and R22a (broken line).

In tables 3, 4 and 5 the results obtained with three other sugary stocks are presented. Progeny R20a was grown from some sugary seeds on the original ear from which the families R21 and R22 were directly descended,

the latter being once removed by self-pollination. Progeny R23a represents a distinct line resulting from a cross between a waxy

TABLE 3

Distribution of waxy and non-waxy seeds obtained on selfing $s_u\ s_u\ W_x\ w_x$ plants in progeny R20a.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R20a-11	541	155	696	22.27±1.11	−2.73
-20	426	109	535	20.37±1.26	−4.63
-29	473	124	597	20.77±1.19	−4.23
-37	449	89	538	16.54±1.26	−8.46
-53	548	132	680	19.41±1.12	−5.59
-56	642	183	825	22.18±1.02	−2.82
-57	597	190	787	24.14±1.04	−0.86
Totals	3676	982	4658	21.08±0.43	−3.92

plant heterozygous for sugary not related to the above stocks and a sugary, non-waxy plant from the commercial variety of sweet corn known as Country Gentleman. R24b and R24c comprise an additional line

TABLE 4

Distribution of waxy and non-waxy seeds obtained on selfing $s_u\ s_u\ W_x\ w_x$ plants in progeny R23a.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R23a- 4	283	75	358	20.95±1.54	−4.05
- 6	553	145	698	20.77±1.10	−4.23
- 7	413	115	528	21.78±1.27	−3.22
- 8	362	66	428	15.42±1.41	−9.58
-11	476	131	607	21.58±1.18	−3.42
-13	201	45	246	18.29±1.86	−6.71
-17	373	127	500	25.40±1.31	+0.40
-18	316	69	385	17.92±1.49	−7.08
-21	334	86	420	20.48±1.42	−4.52
-22	445	111	556	19.96±1.24	−5.04
-27	306	59	365	16.16±1.53	−8.84
-29	429	118	547	21.57±1.25	−3.43
-34	350	85	435	19.54±1.40	−5.46
-36	470	100	570	17.54±1.22	−7.46
-37	256	75	331	22.66±1.60	−2.34
-40	282	74	356	20.79±1.55	−4.21
-12	330	70	400	17.50±1.46	−7.50
-20	334	97	431	22.51±1.41	−2.49
Totals	6513	1648	8161	20.19±0.32	−4.81

differing markedly in habit of growth, time of maturity, shape and size of ear and other characters and presumably carry many distinct factors.

As compared with expectation on the Mendelian basis the selfed ears in progenies R20a and R23a show deficiencies in the waxy class of 3.92 ± 0.43 percent and 4.81 ± 0.32 percent respectively. Families R24b and R24c

TABLE 5

Distribution of waxy and non-waxy seeds obtained on selfing $s_u\ s_u\ W_x\ w_x$ plants in progenies R24b and R24c.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R24b-2	330	53	383	13.84 ± 1.49	-11.16
-5	295	27	322	8.39 ± 1.63	-16.61
-6	242	34	276	12.32 ± 1.76	-12.68
R24c-9	145	11	156	7.05 ± 2.34	-17.95
Totals	1012	125	1137	10.99 ± 0.87	-14.01

are especially noteworthy as exhibiting a much larger discrepancy in the waxy ratio; in this case but 10.99 ± 0.87 percent of the seeds fall in the recessive class.

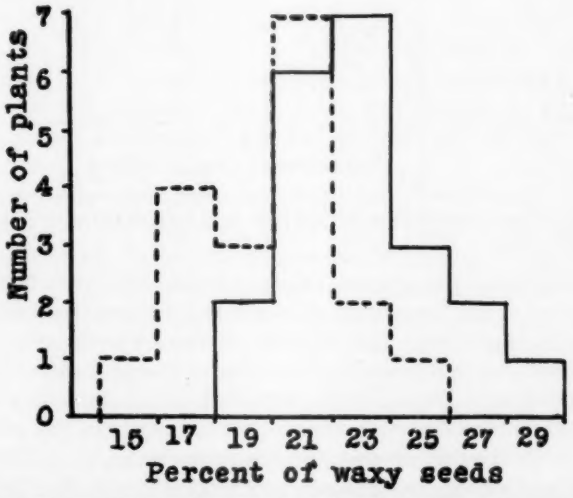

FIGURE 2.—Frequency distributions of plants, with respect to percent of waxy seeds produced on selfing, in the non-sugary progeny R23 (solid line) and the sugary progeny R23a (broken line).

While these latter values cannot be measured against distributions from sib $S_u\ S_u\ W_x\ w_x$ plants their significance may be judged by the results obtained with corresponding stocks heterozygous for both sugary

and waxy which we shall discuss in detail below. The data from the double heterozygous plants given in tables 14, 15 and 17 show—if we may anticipate a conclusion to be established later—that there is little if any difference in the waxy ratio after self-pollinating comparable plants of the constitutions $S_u\ S_u\ W_x\ w_x$ and $S_u\ s_u\ W_x\ w_x$. The non-sugary progenies under consideration are designated R20, R23, R24 and R24a. The percentages of waxy seeds found on self-pollinated ears in these groups are 22.88 ± 0.53, 22.92 ± 0.29 and 22.51 ± 0.43 respectively. The sugary progeny R20a yielded 1.80 ± 0.68 percent fewer waxy grains than the corresponding R20 family which carried the non-sugary factor. The difference in this case is not demonstrably significant but it will be recalled that in the previous year progenies R11 and R13, of which R20a and R20 are duplicates, showed a difference in the same direction in the

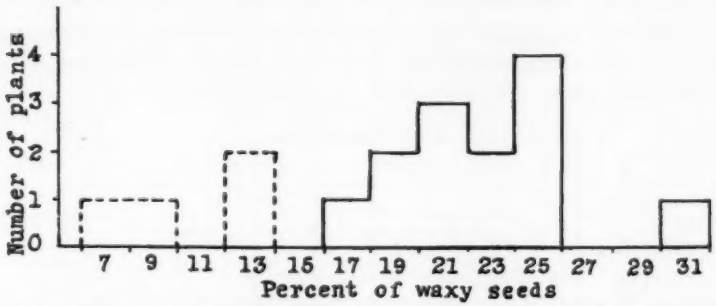

FIGURE 3.—Frequency distributions of plants, with respect to percent of waxy seeds produced on selfing, in the non-sugary progenies R24 and R24a (solid line) and the sugary progenies R24b and R24c (broken line).

proportion of waxy grains amounting to 5.98 ± 0.73 percent. Comparison of the ratios in R23 and R23a shows 2.73 ± 0.43 percent fewer waxy grains on the sugary ears. Such a difference would be expected to occur as a matter of chance in only 1 case in several thousand trials. The odds are overwhelming in the case of the R24 families that the sugary homozygotes produce a smaller proportion of waxy seeds; the difference here amounts to 11.52 ± 0.97 percent. The relations between the waxy ratios in sugary and non-sugary plants in the R21, R22 and R23 groups are shown graphically in figures 1, 2, and 3.

Consideration of these facts shows that waxy heterozygotes which are homozygous for the sugary gene, give, in general, a lower proportion of waxy seeds than similar plants carrying the non-sugary factor. There are relatively wide fluctuations in the waxy ratio from stock to stock due,

presumably, to variations in the residual inheritance. Apparently differences also arise through changes in the environment as demonstrated by the results with the same material grown under the pedigree numbers R11, R13 one year and R20, R20a another. While the difference in the waxy ratios in corresponding sugary and non-sugary stocks in one of the above cases is not clearly significant when examined statistically, there are no exceptions to the rule that the proportion of waxy seeds on the sugary ears is lower; the additional evidence provided by other combinations to be discussed below leaves little room for doubt, however, as to the existence of a real difference between the sugary and non-sugary classes in each of these groups. That the differentiating agent is the sugary gene itself and not some factor associated with it appears probable from the relative regularity in a given stock with which the low waxy ratios follow the sugary gene; this is especially evident in the R24 progenies where the difference is large. This argument finds additional support in the fact that the relationship holds in three pedigreed lines differing in origin.

EFFECT ON THE WAXY RATIO OF THE COMPOSITION OF THE PISTILLATE PARENT WITH RESPECT TO THE SUGARY GENE

In determining the mode of action of the sugary gene in modifying the waxy ratio it is important to know, in the first place, if the effect of the sugary factor is exerted through both the pistillate and staminate parents or in one of these only. On self-pollination, $s_u\ s_u\ W_x\ w_x$ plants give a significantly lower waxy ratio than comparable $S_u\ S_u\ W_x\ w_x$ individuals. Conceivably the large deficiency of waxy seeds in the former case might be the result of a less effective development of $s_u\ w_x$ male gametophytes as compared with $s_u\ W_x$ gametophytes. Does the sugary gene contribute to this deficiency through some effect in the style of the plant or wholly through its action in the pollen? To test this matter crosses were made with a view of studying the effect of the sugary gene in the pistillate and staminate parents separately.

The first of these combinations to be considered is of the type $s_u\ s_u\ w_x\ w_x\ ♀ \times S_u\ S_u\ W_x\ w_x\ ♂$, the pistillate parent only, carrying the sugary gene. Twelve ears were obtained on applying the pollen of $S_u\ S_u\ W_x\ w_x$ plants in progeny R22 to double recessive individuals from 5 different stocks. Table 6 shows the distributions resulting. The average proportion of waxy seeds on these ears was 48.61 percent or 1.39 ± 0.45 percent in defect of Mendelian expectation. While this is possibly a significant departure from a 1:1 ratio it is very similar to the result formerly obtained

(BRINK 1925) in a group of 75 ears from the cross $S_u\ S_u\ w_z\ w_z$ ♀ × $S_u\ S_u\ W_z\ w_z$ ♂. In this latter population 48.25 ± 0.19 percent of the seeds were waxy. It appears, therefore, that the sugary gene acting in the pistillate parent alone does not increase the disparity in the waxy ratio.

Some further evidence on this point is afforded by the following matings. The pollen of a single $S_u\ S_u\ W_z\ w_z$ plant, R22–12, was applied to two waxy plants homozygous for sugary and non-sugary respectively. Of the 454 seeds borne on the ear of the sugary individual, 46.92 ± 1.58 percent were waxy; the ear on the non-sugary plant produced 499 grains of which 46.29 ± 1.51 percent were waxy. The difference, 0.63 ± 2.18 percent, is certainly not significant. The pistillate individuals used were unrelated.

TABLE 6

Distribution of waxy and non-waxy seeds obtained on applying pollen of $S_u\ S_u\ W_z\ w_z$ plants in R22 to double recessives.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R28a- 2×R22- 8	172	168	340	49.41 ± 1.83	−0.59
- 9× - 8	340	352	692	50.87 ± 1.28	+0.87
R35 - 5× -12	268	231	499	46.29 ± 1.51	−3.71
R27 -14× -23	334	287	621	46.22 ± 1.35	−3.79
R28c- 3× -36	217	172	389	44.22 ± 1.71	−5.79
R36 -12× -36	242	241	483	49.90 ± 1.53	−0.10
R28c- 6× -39	127	107	234	45.73 ± 2.20	−4.27
R35 -20× -46	229	243	472	51.48 ± 1.55	+1.48
R37 -12× -48	111	110	221	49.77 ± 2.27	−0.23
R37 -17× -49	312	309	621	49.76 ± 1.35	−0.24
R28b- 5× -55	244	213	457	46.61 ± 1.58	−3.39
R37 - 7× -59	280	287	567	50.62 ± 1.42	+0.62
Totals	2876	2720	5596	48.61 ± 0.45	−1.39

$$\frac{\text{Dev.}}{\text{p. e.}} = \frac{-1.39}{0.45} = 3.09 \quad \text{Odds} = \text{ca } 22{:}1$$

Four $S_u\ S_u\ W_z\ w_z$ plants in R22 were self-pollinated and pollen from them was also used on $s_u\ s_u\ w_z\ w_z$ individuals of diverse breeding. The results obtained are shown in table 7. Since Mendelian theory calls for 25 percent waxy grains in the first case and 50 percent in the second, the numerical results may be put on a comparable basis by halving the observed values in the latter series. If pairs are then formed involving the same pollen parent with the two respective types of pistillate individuals we may test for a possible difference in the waxy ratios following such matings by applying STUDENT'S (1908–09) method. On the average, the adjusted proportion of waxy seeds following the $s_u\ s_u\ w_z\ w_z$ ♀ × $S_u\ S_u\ W_z\ w_z$ ♂

combination is 0.405 percent lower than when the pistillate parent is non-sugary. The standard deviation of the differences between the paired values, however, is 3.285 percent. This gives a value for z of 0.123. One finds on consulting STUDENT's (1915–17) table that the odds corresponding

TABLE 7

The combinations, $S_u\ S_u\ W_z\ w_z$ selfed, and $s_u\ s_u\ w_z\ w_z$ ♀ × $S_u\ S_u\ W_z\ w_z$ ♂, with four R22 plants used as pollen parents.

	$S_u\ S_u\ W_z\ w_z$ SELFED			1	$s_u\ s_u\ w_z\ w_z$ ♀ × $S_u\ S_u\ W_z\ w_z$ ♂					2	
Pedigree number	Non-waxy seeds	Waxy seeds	Total Seeds	Percent waxy	Pedigree number	Non-waxy seeds	Waxy seeds	Total seeds	Percent waxy	ADJUSTED PERCENT	DIFFERENCE 2-1
R22-56	251	99	350	28.29±1.56	R36-13×R22-56	318	289	607	47.61±1.37	23.80	−4.49
-49	286	96	382	25.13±1.49	R37-17×R22-49	312	309	621	49.76±1.35	24.88	−0.25
-48	244	62	306	20.26±1.67	R37-12×R22-48	111	110	221	49.77±2.27	24.88	+4.62
-36	330	102	432	23.61±1.40	R28c-3×R22-36	217	172	389	44.22±1.71	22.11	−1.50
Totals	1111	359	1470	24.42±0.88	Totals	958	880	1838	47.88±0.79	23.94	−0.405

$$z=\frac{M_d}{\sigma_d}=\frac{0.405}{3.285}=0.123 \quad \text{Odds}=\text{ca } 1:1$$

are about 1 to 1. In other words a difference of this magnitude might be expected to occur in one-half the trials as a result of pure chance.

In the above crosses the $S_u\ S_u\ w_z\ w_z$ and $s_u\ s_u\ w_z\ w_z$ stocks used as pistillate parents were not of similar breeding. The criticism might be offered, therefore, that extraneous genetic factors may have obscured a difference arising through the action of the sugary gene in the pistillate

TABLE 8

Distribution of waxy and non-waxy seeds on applying pollen of two $S_u\ S_u\ W_z\ w_z$ plants in R22 to $s_u\ s_u\ W_z\ w_z$ sibs in R22a.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATIONS IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R22a-10×R22-62	334	110	444	24.77±1.39	−0.23
R22a-12×R22-59	324	89	413	21.55±1.44	−3.45
Totals	658	199	857	23.22±1.00	−1.78

individual. The general regularity of the results presented, however, entitles us to considerable confidence in our conclusion.

There is one further bit of evidence, free from the objection noted, which makes it increasingly probable that sugary in the pistillate member of a

pair only does not lower the waxy ratio. Pollen from two $S_u\ S_u\ W_z\ w_z$ plants in R22 was applied to $s_u\ s_u\ W_z\ w_z$ sibs in R22a. The resulting distributions are presented in table 8. The percentage of waxy seeds given in these crosses may be compared with that resulting on self-pollinating non-sugary waxy heterozygotes in progeny R22 as listed in table 1. While the same individuals were not used as pollen parents in the two combinations all the plants used were sibs. $S_u\ S_u\ W_z\ w_z$ selfed yielded 24.42 ± 0.88 percent waxy seeds whereas the cross $s_u\ s_u\ W_z\ w_z$ ♀ $\times S_u\ S_u\ W_z\ w_z$ ♂ gave 23.22 ± 1.00 percent waxy grains. The difference 1.20 ± 1.33 percent is clearly not significant.

We shall next examine the results afforded by combinations of the types $S_u\ S_u\ w_z\ w_z$ ♀ $\times s_u\ s_u\ W_z\ w_z$ ♂ and $s_u\ s_u\ w_z\ w_z$ ♀ $\times s_u\ s_u\ W_z\ w_z$ ♂ with a

TABLE 9

Distribution of waxy and non-waxy seeds on applying pollen of $s_u\ s_u\ W_z\ w_z$ plants in families R21a and R22a to $S_u\ S_u\ w_z\ w_z$ and $s_u\ s_u\ w_z\ w_z$ stocks respectively.

POLLEN PARENT	$S_u\ S_u\ w_z\ w_z$ ♀					$s_u\ s_u\ w_z\ w_z$ ♀					DIFFERENCE
	Number ears	Non-waxy	Waxy	Total	Percent waxy	Number ears	Non-waxy	Waxy	Total	Percent waxy	
R21a	13	3618	2714	6332	42.86 ± 0.42	3	652	340	992	34.27 ± 1.07	−8.59
R22a	15	4554	2343	6897	33.97 ± 0.41	4	1124	642	1766	36.35 ± 0.80	+2.38
Totals	28	8172	5057	13229	38.23	7	1776	982	2758	35.61	−3.10

$$z = \frac{M_d}{\sigma_d} = \frac{-3.10}{5.484} = 0.565 \quad \text{Odds} = \text{ca } 2{:}1$$

view of determining whether the composition of the pistillate parent with respect to the sugary gene exerts a material action when the waxy and non-waxy pollen comes from a sugary plant.

In table 9, the results of applying the pollen of $s_u\ s_u\ W_z\ w_z$ plants in families R21a and R22a to $S_u\ S_u\ w_z\ w_z$ and $s_u\ s_u\ w_z\ w_z$ stocks are shown in summary form. Distributions on individual ears are given in table 10 and 11 and plotted in figure 4.

It will be noted first in table 9 that there is a clearly significant departure from Mendelian proportions in each of the four groups. The deficiencies range from 7.14 ± 0.42 percent to 16.03 ± 0.41 percent, these extremes being shown when the pollen of R21a and R22a, respectively, is applied to $S_u\ S_u\ w_z\ w_z$ plants. The individuals in progenies R21a and R22a are double first cousins; the pistillate individuals on which their pollen

was used were of diverse breeding. Why the R21a combinations with $S_u\ S_u\ w_z\ w_z$ plants regularly gave a higher proportion of waxy seeds than did the same type of cross where R22a was involved, we cannot say. Possibly the two stocks, in spite of their close relationship, differ in regard

TABLE 10

Distribution of waxy and non-waxy seeds on single ears following application of pollen from $s_u s_u W_z w_z$ plants in R21a and R22a to $S_u S_u w_z w_z$ stock.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R25a-27×R21a- 6	215	235	450	52.22±1.59	+2.22
-31× - 4	226	217	443	48.98±1.60	−1.02
-23× - 5	214	161	375	42.93±1.74	−7.07
- 6× - 9	205	153	358	42.74±1.78	−7.26
- 1× - 9	271	188	459	40.96±1.57	−9.04
-14× -23	196	223	419	53.22±1.65	+3.22
R25c-10× -11	315	251	566	44.35±1.42	−5.65
- 6× -14	333	132	465	28.39±1.56	−21.61
- 1× -14	279	177	456	38.81±1.58	−11.19
- 1× -23	368	226	594	38.05±1.38	−11.95
S38 -28× -11	326	303	629	48.17±1.34	−1.83
-135× -14	303	241	544	44.30±1.45	−5.70
-160× -25	367	207	574	36.06±1.41	−13.94
Sub-totals	3618	2714	6332	42.86±0.42	−7.14
R25a-11×R22a- 9	263	146	409	35.70±1.67	−14.30
- 8× - 9	214	137	351	39.03±1.80	−10.97
-18× -14	243	120	363	33.06±1.77	−16.94
R25c-20× -11	352	103	455	22.64±1.58	−27.36
-12× -11	241	163	404	40.35±1.68	−9.65
- 7× -11	377	153	530	28.87±1.46	−21.13
- 4× -14	407	165	572	28.85±1.41	−21.15
S38 -62× - 3	302	178	480	37.08±1.54	−12.92
-98× - 3	402	191	593	32.21±1.38	−17.79
- 2× - 5	345	168	513	32.75±1.49	−17.25
-14× - 9	264	156	420	37.14±1.65	−12.86
-95× -10	222	148	370	40.00±1.75	−10.00
- 2× -11	293	148	441	33.56±1.61	−16.44
-26× -24	328	171	499	34.27±1.51	−15.73
-26× - 9	301	196	497	39.44±1.51	−10.56
Sub-totals	4554	2343	6897	33.97±0.41	−16.03
Totals	8172	5057	13229	38.23	−11.77

to some accessory factors which, under certain conditions, markedly affect the waxy ratio. The anomolous value shown by the R21a group in the one combination makes the entire body of data difficult to interpret. We cannot justifiably apply the ordinary probable error formula to the

totals from the $S_u\ S_u\ w_x\ w_x$ matings since the distributions on ears resulting from R21a and R22a pollen clearly do not form a homogeneous

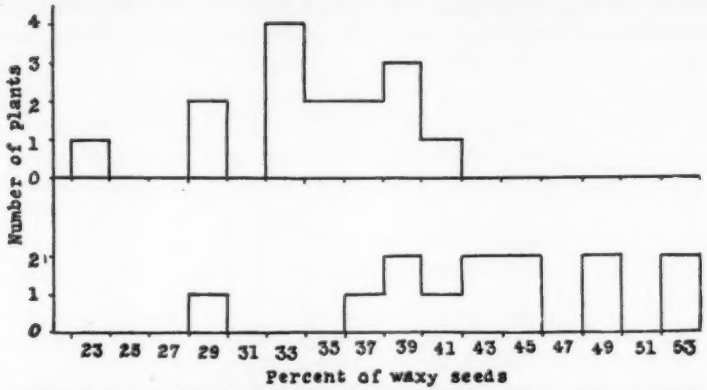

FIGURE 4.—Frequency distributions of plants, with respect to percent of waxy seeds produced in the backcross $S_u\ S_u\ w_x\ w_x\ ♀ \times s_u\ s_u\ W_x\ w_x ♂$. The polygons above relate to the ears obtained on using pollen from progeny R22a and those below to the ears resulting from the application of pollen from the sib progeny R21a.

group. If we compare the results obtained on crossing each of the staminate groups with the two respective pistillate stocks we find, in the case of R21a, that the $s_u\ s_u\ w_x\ w_x$ combination shows 8.59 ± 1.15 percent fewer

TABLE 11

Distribution of waxy and non-waxy seeds on single ears following application of pollen from $s_u s_u W_x w_x$ plants in R21a and R22a to $s_u s_u w_x w_x$ stocks.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R37 -10×R21a- 8	162	98	260	37.69 ± 2.09	-12.31
-16× - 9	142	82	224	36.61 ± 2.25	-13.39
R28 - 2× - 8	348	160	508	31.50 ± 1.50	-18.50
Sub-totals	652	340	992	34.27 ± 1.07	-15.73
R28 - 4×R22a-14	311	171	482	35.48 ± 1.54	-14.52
R28b- 8× -24	364	212	576	36.81 ± 1.40	-13.19
-10× -25	234	98	332	29.52 ± 1.85	-20.48
-17× - 5	215	161	376	42.82 ± 1.74	-7.18
Sub-totals	1124	642	1766	36.35 ± 0.80	-13.65
Totals	1776	982	2758	35.61	-14.39

waxy grains than the other type of mating. This difference is certainly significant statistically. The R22a crosses, on the other hand, reveal a

difference in the opposite direction of 2.39 ± 0.90, which, however, might be due to chance.

Evaluation of these data by STUDENT's method shows that it would be unsafe to conclude that a significant difference in the waxy ratio incident upon a difference in composition of the pistillate plants is indicated here. The last column in table 9 shows that the average difference in percentage of waxy seeds in the two types of crosses is -3.10. But the standard deviation of the differences is 5.48 giving z the low value of 0.565. This value of z corresponds to a probability of only 1.86:1 that the ratio is actually lower in the case of the $s_u\ s_u\ w_x\ w_x$ combination. Considering the facts as a whole, a definite conclusion that a significant difference is or is not shown seems unwarranted. It should be emphasized, however, that the proportions of waxy seeds in both types of crosses are, on the average, markedly lower than those observed when pollen of $S_u\ S_u\ W_x\ w_x$ plants is employed.

TABLE 12

Distribution of waxy and non-waxy seeds on applying pollen of the same $s_u s_u W_x w_x$ plants from R21a and R22a to both $S_u S_u w_x w_x$ and $s_u s_u w_x w_x$ stocks.

$S_u S_u w_x w_x$ ♀						$s_u s_u w_x w_x$ ♀					
Pedigree number	Non-waxy	Waxy	Total	Percent waxy		Pedigree number	Non-waxy	Waxy	Total	Percent waxy	DIFFERENCE
R25a- 6×R21a- 9	205	153	358	42.74	41.73	R37 -16×R21a- 9	142	82	224	36.61	−5.12
- 1×R21a- 9	271	188	459	40.96							
S38 - 2×R22a- 5	345	168	513	32.75		R28b-17×R22a- 5	215	161	376	42.82	+10.07
R25a-18×R22a-14	243	120	363	33.06	30.48	R28 - 4×R22a-14	311	171	482	35.48	+5.00
R25c- 4×R22a-14	407	165	572	28.85							
S38 - 26×R22a-24	328	171	499	34.27		R28b- 8×R22a-24	364	212	576	36.81	+2.54
Totals	1799	965	2764	34.91 ± 0.64			1032	626	1658	37.76 ± 0.83	+3.12

$$z = \frac{M_d}{\sigma_d} = \frac{+3.12}{5.478} = 0.569 \quad \text{Odds ca 4:1}$$

The data assembled in table 12 furnish somewhat more critical evidence on the question at issue. In this group of crosses pollen from single $s_u\ s_u\ W_x\ w_x$ plants was applied to both pistillate types. This enables us to eliminate any major variation occasioned by the staminate parents. Again, however, the homogeneity of the data is doubtful and the use of the ordinary probable error formula in the case of the totals is of questionable value. A more reliable measure of the significance of the deviations shown is afforded, perhaps, by the pairing method and consideration of the average difference in relation to the standard deviation of the differ-

ences. As shown in the last column of table 12 the average difference in percent of waxy seeds following crosses with the two types of pistillate individuals, is 3.12, the ears borne on the double recessive plants giving the higher ratio of waxy grains. It will be noted that the sign of the net difference is opposite to that in the case considered above. The standard deviation of the differences is 5.478 percent giving a value of z of 0.569. The corresponding odds are about 4:1; or in about 1 trial in 5 we should expect such a difference to occur by chance. These data afford no evidence, therefore, of a difference in the waxy ratio when $s_u\ s_u\ W_z\ w_z$ plants are crossed with sugary and non-sugary pistillate stocks.

Summarizing the results of the experiments on the effect of the sugary gene in the pistillate member of a pair on the waxy ratio we find:

1. When pollen from a non-sugary plant heterozygous for waxy ($S_u\ S_u\ W_z\ w_z$) is applied to the double recessive ($s_u\ s_u\ w_z\ w_z$) a small deficiency in the waxy class amounting to about 1.5 percent occurs. This discrepancy in the waxy ratio is no greater than that found in $w_z\ w_z\ ♀ \times W_z\ w_z\ ♂$ crosses not involving the sugary factor.

2. The combination $s_u\ s_u\ w_z\ w_z\ ♀ \times s_u\ s_u\ W_z\ w_z\ ♂$ gives a large deficiency in the waxy class of roughly 15 percent.

3. The cross $S_u\ S_u\ w_z\ w_z\ ♀ \times s_u\ s_u\ W_z\ w_z\ ♂$ likewise results in a large minus deviation in the waxy class of about 15 percent.

The sugary gene in homozygous condition in the pistillate member of a pair only does not, therefore, depress the waxy ratio. When the pollen parent is homozygous for the sugary factor ($s_u\ s_u\ W_z\ w_z$) a large deficiency of waxy seeds results in combinations with waxy plants whether these be sugary or non-sugary. These findings point to the conclusion that the sugary gene exerts its disturbing influence on the waxy ratio through some action on the pollen and that the composition of the pistillate member of a mating with regard to this factor is of little, possibly no significance.

ADDITIONAL EVIDENCE FROM BACKCROSSES THAT THE SUGARY GENE DEPRESSES THE WAXY RATIO

Twelve ears were obtained on applying pollen from R23a ($s_u\ s_u\ W_z\ w_z$) to $S_u\ S_u\ w_z\ w_z$ plants. The distributions of waxy and non-waxy seeds on individual ears are given in table 13. The average proportion of waxy seeds resulting was 35.95 ± 0.48 percent. The large deficiency in the waxy class amounting to 14.05 ± 0.48 percent is of the same order of magnitude as those obtained in similar combinations with the other stocks.

In contrast with these results from sugary plants in R23a we find, as shown at the end of table 18, an average of 47.16 ± 0.97 percent waxy seeds on two ears following the application of pollen from $S_u\ s_u\ W_x\ w_x$

TABLE 13

Distribution of waxy and non-waxy seeds on applying pollen of $s_u s_u W_x w_x$ plants in R23a to $S_u S_u w_x w_x$ individuals.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN THE WAXY CLASS IN PERCENT
	Non-waxy	Waxy	Total		
R25a-18×R23a- 7	229	160	389	41.13 ± 1.71	-8.87
- 4× -11	266	171	437	39.13 ± 1.61	-10.87
-13× -16	211	85	296	28.72 ± 1.96	-21.28
-26× -17	180	150	330	45.45 ± 1.86	-4.55
-12× -36	256	121	377	32.10 ± 1.74	-17.90
-15× -37	194	67	261	25.67 ± 2.09	-24.33
R25b- 4× -13	287	135	422	31.99 ± 1.64	-18.01
R25c- 3× -21	254	163	417	39.09 ± 1.65	-10.91
-17× -21	255	141	396	35.61 ± 1.69	-14.39
-18× -29	323	221	544	40.62 ± 1.45	-9.38
-23× -34	349	267	616	43.34 ± 1.36	-6.66
R25d- 1× - 4	399	117	516	22.67 ± 1.48	-27.33
Totals	3203	1798	5001	35.95 ± 0.48	-14.05

plants in R23 to double recessives. Similarly two ears obtained on applying R23 pollen to R23a plants, a combination of the type $s_u\ s_u\ W_x\ w_x$ ♀ $\times S_u\ s_u\ W_x\ w_x$ ♂, contained 26.16 ± 1.19 percent waxy grains (table 16).

The progenies R23 and R23a are of different breeding than any of the other stocks used as pollen parents in these experiments and it is important to have this confirmatory evidence that the sugary gene operates to depress the waxy ratio in this group as in the other cases discussed.

THE WAXY RATIO IN THE SUGARY AND NON-SUGARY CLASSES FOLLOWING THE USE OF THE DOUBLE HETEROZYGOTE, $S_u\ s_u\ W_x\ w_x$, AS THE STAMINATE PARENT

It has been shown above that waxy heterozygotes, homozygous for the non-sugary gene, when used as male parents with the double recessive type give approximately 48 percent of waxy seeds; waxy heterozygotes homozygous for sugary, on the other hand, in combination with the same pistillate form produce only about 35 percent of waxy grains. If the sugary gene exercises its initial differential effect in the male gametophyte after this has become independent of the parent sporophyte, that is, in the mature pollen grain or in the pollen tube, we should expect, following the

cross $s_u\ s_u\ w_x\ w_x$ ♀ $\times S_u\ s_u\ W_x\ w_x$ ♂, about 48 percent waxy seeds in the non-sugary class and 35 percent in the sugary group. The double heterozygote used as the staminate parent produces, of course, four classes of pollen grains, namely, $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ and $s_u\ w_x$. The first two types correspond to those formed by $S_u\ S_u\ W_x\ w_x$ plants and the other two classes are identical, so far as nuclear composition is concerned, with the

TABLE 14

Distribution of waxy seeds in the sugary and non-sugary classes on selfing double heterozygotes in progenies R20, R21, and R22.

PROGENY NUMBER	NON-SUGARY				SUGARY				DIFFERENCE
	Non-waxy	Waxy	Total	Percent waxy	Non-waxy	Waxy	Total	Percent waxy	
R20- 1	231	68	299	22.74±1.69	84	21	105	20.00±2.85	−2.74
- 2	292	78	370	21.08±1.52	102	42	144	29.17±2.43	+8.09
-12	333	120	453	26.49±1.37	128	26	154	16.99±2.35	−9.50
-13	252	76	328	23.18±1.61	90	19	109	17.43±2.80	−5.75
-22	253	98	351	27.92±1.56	86	33	119	27.73±2.68	−0.19
-23	353	77	430	17.91±1.41	128	34	162	20.99±2.29	+3.08
Sub-totals	1714	517	2231	23.17±0.62	618	175	793	22.07±1.04	
R21- 2	463	163	626	26.04±1.17	157	53	210	25.24±2.01	−0.80
- 3	338	126	464	27.16±1.36	102	26	128	20.31±2.58	−6.85
- 5	214	71	285	24.91±1.73	73	17	90	18.89±3.08	−6.02
- 9	211	79	290	27.24±1.71	78	18	96	18.75±2.98	−8.49
-24	297	100	397	25.19±1.47	90	36	126	28.57±2.60	+3.38
-32	252	88	340	25.88±1.58	82	25	107	23.36±2.82	−2.52
-37	349	126	475	26.53±1.34	123	39	162	24.07±2.29	−2.46
-61	248	79	327	24.16±1.61	68	30	98	30.61±2.95	+6.45
Sub-totals	2372	832	3204	25.97±0.52	773	244	1017	23.99±0.92	
R22- 1	109	39	148	26.35±2.40	50	10	60	16.67±3.77	−9.68
-26	341	93	434	21.43±1.40	113	35	148	23.65±2.40	+2.22
-45	243	64	307	20.85±1.67	92	28	120	23.33±2.67	+2.48
-47	210	64	274	23.36±1.76	72	26	98	26.53±2.95	+3.17
Sub-totals	903	260	1163	22.36±0.86	327	99	426	23.24±1.41	−1.45

$$z=\frac{M_d}{\sigma_d}=\frac{-1.45}{5.29}=0.274 \quad \text{Odds}=6:1$$

pollen formed by $s_u\ s_u\ W_x\ w_x$ individuals; if genetic constitution of the gametophyte alone is the determining factor the double heterozygote should give markedly different waxy ratios in the sugary and non-sugary classes. It is a remarkable fact that such a difference does not eventuate. Ears from the cross $s_u\ s_u W_x w_x$ ♀ $\times S_u\ s_u\ W_x\ w_x$ ♂ or from self-pollinated $S_u\ s_u\ W_x\ w_x$ individuals show about the same proportion of waxy seeds in the S_u and s_u classes.

A considerable body of evidence has been accumulated bearing on this relation for the point is an important one. STUDENT's method of analysis lends itself admirably to the evaluation of these data, where correlation exists between the ratio of waxy seeds in the sugary and non-sugary classes from single ears, and has been employed throughout. The pairs formed consist, naturally, of the percentages of waxy seeds in the two respective

TABLE 15

Distribution of waxy seeds in the sugary and non-sugary classes on selfing double heterozygotes in progeny R23.

PROGENY NUMBER	NON-SUGARY				SUGARY				DIFFERENCE
	Non-waxy	Waxy	Total	Percent waxy	Non-waxy	Waxy	Total	Percent waxy	
R23- 5	245	71	316	22.47±1.64	143	40	183	21.86±2.16	−0.61
- 7	204	60	264	22.73±1.80	76	24	100	24.00±2.92	+1.27
-11	309	92	401	22.94±1.46	109	32	141	22.67±2.46	−0.27
- 3	321	103	424	24.29±1.42	104	31	135	22.96±2.51	−1.33
-18	350	93	443	20.99±1.39	114	38	152	25.00±2.37	+4.01
-20	305	101	406	24.88±1.45	100	28	128	21.87±2.58	−3.01
-25	362	98	460	21.30±1.36	125	34	159	21.38±2.32	+0.08
-29	260	81	341	23.75±1.58	86	29	115	25.22±2.72	+1.47
-32	293	118	411	28.71±1.44	125	35	160	21.87±2.31	−6.84
-34	382	90	472	19.07±1.34	145	43	188	22.87±2.13	+3.80
-36	299	83	382	21.73±1.49	102	20	122	16.39±2.64	−5.34
-33	305	113	418	27.03±1.43	108	41	149	27.52±2.39	+0.49
-41.5	238	86	324	26.54±1.62	90	15	105	14.29±2.85	−12.25
-43	311	103	414	24.88±1.43	124	37	161	22.98±2.30	−1.90
-49	194	60	254	23.62±1.83	93	10	103	9.71±2.88	−13.91
-57	235	66	301	21.93±1.68	97	22	119	18.49±2.68	−3.44
-12	254	70	324	21.60±1.62	68	18	86	20.93±3.15	−0.67
-14	215	60	275	21.82±1.76	69	11	80	13.75±3.26	−8.07
-51	116	52	168	30.95±2.25	55	17	72	23.61±3.44	−7.34
-52	181	49	230	21.30±1.93	54	16	70	22.86±3.49	+1.56
-59	173	49	222	22.07±1.96	50	18	68	26.47±3.54	+4.40
Totals	5552	1698	7250	23.42±0.34	2037	559	2596	21.53±0.57	−2.281

$$z=\frac{M_d}{\sigma_d}=\frac{-2.281}{4.938}=0.462 \quad \text{Odds}=34:1$$

classes on ears segregating for sugary. Since we are comparing two distributions on the same ear we should expect fluctuations due to accessory circumstances to be reduced to a minimum affording our results a considerable degree of accuracy.

The first group of data to be considered comprises the distributions from self-pollinated plants of the constitution $S_u\ s_u\ W_z\ w_z$ in progenies R20, R21 and R22. These families are closely related, R21 and R22

having been derived from sibs of R20 by self-pollination. The results are given in detail in table 14. In the non-sugary class 24.39 percent of the seeds are waxy as compared with 23.17 percent in the sugary group.

TABLE 16

Distribution of waxy seeds in the sugary and non-sugary classes following crosses of the type $s_u s_u W_z w_z$ ♀ × $S_u s_u W_z w_z$ ♂ *with R23a and R23 plants.*

PROGENY NUMBER	NON-SUGARY				SUGARY			
	Non-waxy	Waxy	Total	Percent waxy	Non-waxy	Waxy	Total	Percent waxy
R23a- 3×R23-36	88	21	109	19.27±2.80	100	32	132	24.24±2.54
-10× -18	134	51	185	27.57±2.15	124	54	178	30.34±2.19
Totals	222	72	294	24.49±1.70	224	86	310	27.74±1.66

Examining the percentages of waxy grains in the sugary and non-sugary classes in the group of 18 ears by STUDENT'S method we find an average difference of 1.45 percent, the sugary class showing the lower ratio

TABLE 17

Distribution of waxy seeds in the sugary and non-sugary classes obtained on selfing double heterozygotes in progenies R24 and R24a.

PROGENY NUMBER	NON-SUGARY				SUGARY				DIFFERENCE
	Non-waxy	Waxy	Total	Percent waxy	Non-waxy	Waxy	Total	Percent waxy	
R24 - 1	211	61	272	22.43±1.77	70	22	92	23.91±3.04	+1.48
- 1	277	96	373	25.74±1.51	65	19	84	22.62±3.19	−3.12
R24a- 1	203	45	248	18.15±1.85	53	18	71	25.35±3.47	+7.20
- 5	116	59	175	33.71±2.21	38	13	51	25.49±4.09	−8.22
-10	261	85	346	24.57±1.57	77	22	99	22.22±2.90	−2.35
-14	217	50	267	18.73±1.79	63	21	84	25.00±3.19	+6.27
-15	290	57	347	16.43±1.57	80	21	101	20.79±2.91	+4.36
-20	188	54	242	22.31±1.88	55	18	73	24.66±3.42	+2.35
-24	148	36	184	19.57±2.15	41	13	54	24.07±3.97	+4.50
-27	242	63	305	20.66±1.67	44	14	58	24.14±3.83	+3.48
-31	196	48	244	19.67±1.87	58	14	72	19.44±3.44	−0.23
-34	213	60	273	21.78±1.77	68	31	99	31.31±2.93	+9.53
-37	198	61	259	23.55±1.81	59	25	84	29.76±3.19	+6.21
Totals	2760	775	3535	21.92±0.49	771	251	1022	24.56±0.91	

$$z = \frac{M_d}{\sigma_d} = \frac{+2.420}{4.705} = 0.514 \quad \text{Odds} = \text{ca } 18{:}1$$

The standard deviation of the differences is 5.29 percent giving z a value of 0.274. This gives odds of but 6 to 1, which indicates that very probably the difference is not significant.

In the unrelated progeny R23, 21 $S_u\, s_u\, W_z\, w_z$ plants were self-pollinated. The distributions obtained on the single ears are listed in table 15. The respective net percentages of waxy seeds in the non-sugary and sugary classes in this case are 23.42 and 21.53. The mean difference again reveals a lower proportion of waxy seeds in the sugary class amounting to 2.281

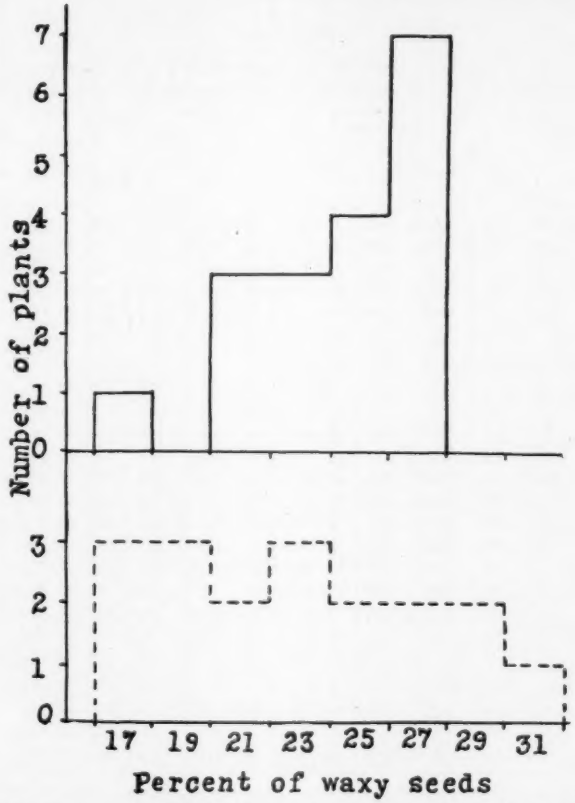

FIGURE 5.—Frequency distributions of percentages of waxy seeds in the non-sugary (solid line) and the sugary (broken line) classes on selfing $S_u\, s_u\, W_z\, w_z$ plants in progenies R24 and R24a.

percent. The standard deviation of the differences is 4.938 percent and the value of z, 0.462. In this case the odds are about 34:1 that the proportion of waxy seeds in the sugary class is significantly lower than that in the non-sugary group.

A little further evidence on the behavior of progeny R23 is afforded by the results of two crosses with sib plants in family R23a. The distributions resulting from these combinations of the type $s_u\ s_u\ W_x\ w_x$ ♀ $\times S_u\ s_u\ W_x\ w_x$ ♂ are given in table 16. It will be seen that in the case of both ears the waxy ratio is higher in the sugary class, a result contrary to that afforded by self-pollinating R23 plants.

TABLE 18

Distribution of waxy seeds in the sugary and non-sugary classes obtained on backcrossing $S_u s_u W_x w_x$ plants in progenies R21, R22, and R23 to the double recessive used as the pistillate parent.

PEDIGREE NUMBER	NON-SUGARY				SUGARY				DIFFERENCE
	Non-waxy	Waxy	Total	Percent waxy	Non-waxy	Waxy	Total	Percent waxy	
R27-11×R21-38	169	162	331	48.94±1.85	195	163	358	45.53±1.78	−3.41
R28- 7× -52	129	97	226	42.92±2.24	110	82	192	42.71±2.43	−0.21
R36- 3× - 9	129	137	266	51.50±2.07	118	109	227	48.02±2.24	−3.48
R38-11× -22	209	153	362	42.27±1.77	196	172	368	46.74±1.76	+4.47
R38- 3× -24	136	100	236	42.37±2.20	133	125	258	48.45±2.10	+6.08
Sub-totals	772	649	1421	45.67±0.89	752	651	1403	46.40±0.90	
R27- 6×R22- 5	210	170	380	44.74±1.73	217	196	413	47.46±1.66	+2.72
- 1× -11	177	176	353	49.86±1.79	192	198	390	50.77±1.71	+0.91
-15× -26	126	141	267	52.81±2.06	132	140	272	51.47±2.04	−1.34
-13× -27	183	137	320	42.81±1.89	186	148	334	44.31±1.85	+1.50
-17× -44	173	168	341	49.27±1.83	153	147	300	49.00±1.95	−0.27
R28- 5× -14	218	195	413	47.22±1.66	182	169	351	48.15±1.80	+0.93
R28c-9× -30	60	56	116	48.27±3.13	78	58	136	42.65±2.89	−5.62
R36-21× -47	112	111	223	49.78±2.26	104	103	207	49.76±2.34	−0.02
R27- 7× -54	79	101	180	56.11±2.51	108	224	332	67.47±1.85	+11.36
R37- 7× - 4	86	97	183	53.01±2.49	76	85	161	52.79±2.66	−0.22
-19× - 7	93	66	159	41.51±2.67	85	58	143	40.56±2.82	−0.95
-17× -18	74	82	156	52.56±2.70	105	82	187	43.85±2.47	−8.71
-18× -29	77	60	137	43.80±2.88	81	86	167	51.50±2.61	+7.70
- 3× -45	85	67	152	44.08±2.74	84	72	156	46.15±2.70	+2.07
Sub-totals	1753	1627	3380	48.13±0.58	1783	1766	3549	49.76±0.57	
R28a-4×R23-32	133	138	271	50.92±2.05	134	119	253	47.04±2.12	−3.88
- 5 -58	169	154	323	47.66±1.88	207	163	370	44.05±1.75	−3.61
Sub-totals	302	292	594	49.16±1.38	341	282	623	45.26±1.35	

$$z=\frac{M_d}{\sigma_d}=\frac{+0.287}{4.48}=0.064 \quad \text{Odds} < 2{:}1$$

A further group of selfed ears of this type was obtained in progenies R24 and R24a. The distributions are given in table 17 and shown graphically in figure 5. It will be recalled that the $s_u\ s_u\ W_x\ w_x$ sibs of these plants, in progenies R24b and R24c, when self-pollinated gave very low waxy ratios amounting, on the average, to 10.99 percent. The $S_u\ s_u\ W_x$

w_x plants of the same breeding show 21.92 percent and 24.56 percent waxy seeds in the non-sugary and sugary classes respectively. Calculating the average of the differences for the 13 selfed ears from the double heterozygotes we find that the proportion of waxy seeds in the sugary

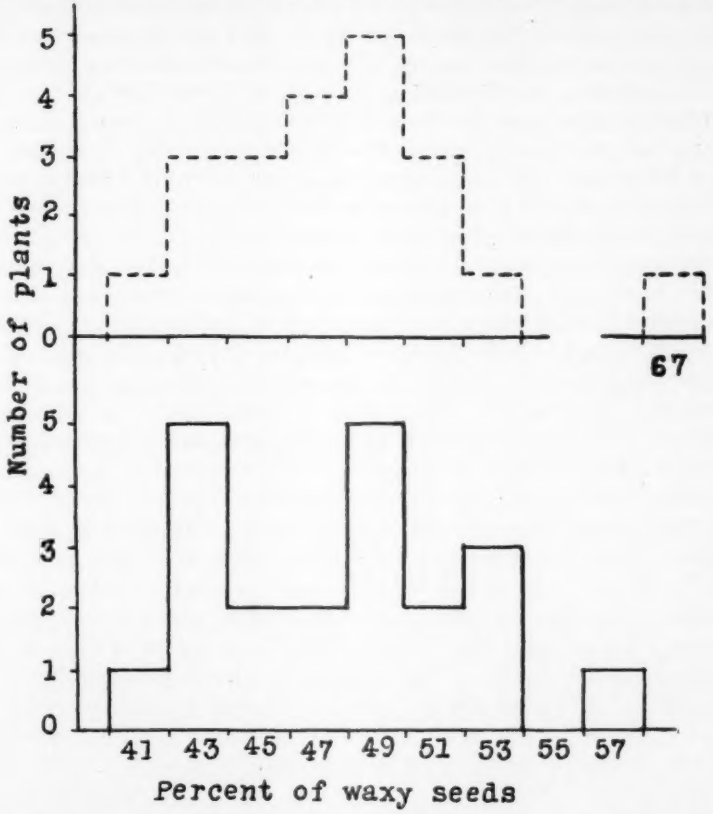

FIGURE 6.—Frequency distributions of percentages of waxy seeds in the non-sugary (solid line) and the sugary (broken line) classes following the combination $s_u\ s_u\ w_x\ w_x ♀ \times S_u\ s_u\ W_x\ w_x ♂$; plants in progenies R21. R22 and R23 were used as pollen parents.

class is in excess by 2.420 percent. Since the standard deviation is 4.705 percent, z equals 0.514. This gives odds of about 19:1 that the two groups differ significantly.

Analysis of the data afforded by the first of these three main groups of $S_u\ s_u\ W_x\ w_x$ ears shows a somewhat lower percentage of waxy grains in

the sugary class; in the third group the difference is in the opposite direction. Probably in neither of these cases, however, is the difference significant. In the second group comprising progeny R23 there does appear to be a significantly lower waxy ratio in the sugary class. Two plants in R23, numbers 41.5 and 49, show relatively large differences in proportion of waxy seeds in the sugary and non-sugary phenotypes amounting, respectively, to -12.25 percent and -13.91 percent. In view of the other findings it may be fairly questioned, in the case of these two individuals, whether some other factor has not entered in to disturb the waxy ratio. If the two items are omitted the mean difference is reduced to -1.144 percent and the standard deviation of the differences becomes 3.648 percent. This gives z the value 0.314 corresponding to odds of but 9:1 that the difference is a significant one.

A total of 21 ears was obtained in backcrosses of the type $s_u\ s_u\ w_z\ w_z$ ♀ $\times S_u\ s_u\ W_z\ w_z$ ♂. The pollen parents involved in 19 cases came from progenies R21 and R22 and in the remaining 2 cases from R23. The double recessive pistillate individuals used fall into 3 groups. Progenies R27, R28, R28a, R28b and R36 have a common origin identical with that of R21 and R22; R28c and R37 belong to distinct lines. If there is an inherent difference in the effectiveness of the four classes of male gametophytes arising from $S_u\ s_u\ W_z\ w_z$ plants we should expect it to be manifested in these backcrosses. The distributions from single ears are presented in table 18 and shown in the form of frequency polygons in figure 6. Examination of the group as a whole shows a remarkably close agreement in the ratio of waxy seeds in the sugary and non-sugary classes; the non-sugary group has a net percentage of 47.60 waxy grains and the sugary group, 48.41 percent. When examined by STUDENT's method the sugary class is found to have on the average, 0.287 percent fewer waxy grains, a difference which is clearly not significant in view of the standard deviation of the differences which amounts to 4.48 percent. The value of z is only 0.064 and is below the range of STUDENT's table of odds. The chances are less than 2:1, therefore that the difference is a significant one.

THE SUGARY RATIO WHEN $S_u\ s_u\ W_z\ w_z$ PLANTS ARE USED AS STAMINATE PARENTS

If there were a tendency for $s_u\ w_z$ male gametophytes to be handicapped to a greater extent than the other types of gametophytes arising from $S_u\ s_u\ W_z\ w_z$ plants we should expect a deficiency in the sugary class as well as in the waxy in $S_u\ s_u\ W_z\ w_z$ ♀ $\times S_u\ s_u\ W_z\ w_z$ ♂ and $s_u\ s_u\ w_z\ w_z$ ♀ $\times S_u\ s_u\ W_z\ w_z$ ♂ combinations. Examination of the data in tables 14, 15, 17

and 18 shows no clear evidence of such a deficiency. In progenies R20, R21 and R22 there is a net excess of 27.5 ± 27 seeds as compared with Mendelian expectation. In R23 an excess in the sugary class again results amounting to 134.5 ± 29 grains; this deviation is over 4.6 times its probable error and appears significant, indicating further that there may be some unknown factor operating in progeny R23 to distort the sugary and waxy ratios. In progenies R24 and R24a we find a net deficiency in the sugary class of 117.25 ± 20 seeds. This result is clearly not in conformity with Mendelian expectation. R24 and R24a produced a higher proportion of waxy seeds in the sugary class than in the non-sugary (see table 17) which would indicate that the low sugary ratio is not a result of the less effective development of $s_u\ w_x$ male gametophytes. The distributions in table 18 resulting from backcrosses of the double heterozygote to the double recessive show a net excess of 90 ± 35 seeds in the sugary class. This deviation does not appear statistically significant.

While there are irregularities in the sugary ratios in certain of these stocks they do not bear any evident relation to the proportion of waxy seeds; the deviations in the sugary class are not such as would result from a less effective development of $s_u\ w_x$ male gametophytes as compared with the other three classes produced by $S_u\ s_u\ W_x\ w_x$ plants.

DISCUSSION

The fact of particular interest emerging from these experiments is that the two classes of pollen $s_u\ W_x$ and $s_u\ w_x$ lead to different waxy ratios according to the type of sporophyte from which the pollen grains arise; if the $W_x\ w_x$ plant is homozygous for the sugary gene a large deficiency in the proportion of waxy seeds results while if the dominant non-sugary factor is present along with the sugary gene ($S_u\ s_u$) the deviation from Mendelian expectation in the sugary class is only about one-seventh as large. The evidence shows that the presence of the sugary gene in the pistillate parent is not important, $S_u\ S_u$ or $S_u\ s_u$ and $s_u\ s_u$ individuals giving the same waxy ratios. We have to do, then, with an effect on the male gametophyte which is expressed independently of the genetic composition of the stylar tissue with which the gametophyte becomes associated.

There are really two problems to be distinguished in this situation, the character of the handicap which sugary sporophytes place upon waxy male gametophytes and, secondly, when and how this handicap operates to depress the waxy ratio.

It should be noted that waxy and non-waxy seeds resulting from the use of pollen from homozygous sugary individuals are in no obvious way different from the same classes descended from heterozygous non-sugary plants. The new individuals show no sign of any weakness such as might supposedly result if the "potency" of the male gametes participating in fertilization had been impaired through having come from an $s_u\ s_u$ plant. So far as their genetic organization is concerned, therefore, $s_u\ W_x$ and $s_u\ w_x$ sperm delivered to the eggs are identical whatever the composition of the sporophyte from which they are descended. There is no evidence of a nuclear difference then, in the same classes of pollen produced by pure sugary and heterozygous sugary plants.

It seems more probable that a cytoplasmic change is involved. One might suppose that under the sustained action of the sugary and non-sugary genes different kinds of cytoplasm are formed in the two respective kinds of sporophytes. For convenience in discussion we might call the cytoplasm formed in non-sugary plants "normal" and that in sugary plants, for want of a more precise term, "modified." At sporogenesis these respective types of cytoplasm are passed on to the gametophytes; in microsporogenesis the cytoplasm of each spore mother cell is partitioned among four microspores which develop quickly into the simple male gametophytes. Any peculiarity of the cytoplasm of the sporophyte, therefore, might well characterize the young gametophytes also. The $s_u\ W_x$ and $s_u\ w_x$ haploid nuclei arising from $S_u\ s_u\ W_x\ w_x$ plants would be accompanied by normal cytoplasm since this sporophyte carries the dominant non-sugary gene; the same classes of gametophytic nuclei formed by $s_u\ s_u\ W_x\ w_x$ individuals would be associated with modified cytoplasm. It is necessary to assume further that the initial differences in the cytoplasm persist for a certain interval in the haploid generation. In the presence of normal cytoplasm waxy male gametophytes make nearly as effective development as non-waxy individuals; if, on the other hand, the cytoplasm of the microspore is derived from a sugary sporophyte, that is, is of the modified type, waxy gametophytes are handicapped in development to a much greater extent as compared with the alternative non-waxy class.

Accepting this as a working hypothesis we may inquire as to the probable nature of the cytoplasmic modification in the case of sugary plants. The sugary gene exerts a profound effect on the character of the endosperm reserves, these being changed from mainly starch to a complex mixture of carbohydrates. The two types of grains, non-sugary and sugary, are produced in a definite proportion on the ears of segregating plants.

This latter fact shows that in the seed the determining factor is nuclear composition ultimately at least, and not the character of the cytoplasm contributed by the parent. It must be borne in mind, however, that embryo and endosperm represent many cell generations and are developed during an interval of time sufficiently long probably to permit the original character of the cytoplasm to be modified according to the composition of the nucleus.

It is quite possible that the composition of the pollen is also influenced by the sugary gene. But the haploid nuclei are associated with the cytoplasm of the gametophyte for a relatively brief period of time; the interval is too short, perhaps, for the extra-nuclear constituents to conform entirely to the character of the chromosome complement present. The sugary gene may modify the composition of the pollen, therefore, only indirectly through its previous action on the cytoplasm of the sporophyte. This may account for the fact that $s_u\ W_x$ and $s_u\ w_x$ pollen grains from $S_u\ s_u\ W_x\ w_x$ and $s_u\ s_u\ W_x\ w_x$ plants function differently. In the former case the cytoplasm accompanying $s_u\ W_x$ and $s_u\ w_x$ microspore nuclei has been formed under the action of the dominant non-sugary factor; in $s_u\ s_u\ W_x\ w_x$ plants, on the other hand, the sugary gene has operated throughout the sporophyte generation and the cytoplasm passed on to the microspore is already in equilibrium with this nuclear condition, so to speak.

Starch formation takes place in the amyloplastids, definitely organized, extra-nuclear cell constituents. It is clear, however, from a consideration of the effects of such genes as sugary and waxy in maize that starch synthesis is not accomplished by the plastid functioning as an independent unit; the process is influenced by the character of the nucleus as well. We are not prepared at the present time to enter into a discussion of the rôle of the nucleus in amyloplastid activity although it would not be surprising to find that the relationship which exists between these cell organs holds the secret of the present results. It might reasonably be supposed that the immediate basis for the production of the peculiar reserves characteristic of sugary seeds lies in some abnormal condition in the plastid determined during the development of the latter. The point is obscure but it may be that no new plastids or only a comparatively few are formed in the male gametophyte, its complement of these bodies having been derived wholly or in large part from the parent sporophyte. If this were so we might expect a difference in metabolic activity between pollen from sugary and non-sugary plants but not between S_u and s_u gametophytes arising from the heterozygote.

There is nothing to be gained at the present stage of this investigation, perhaps, by speculating as to the immediate nature of the change effected in $s_u\ w_x$ pollen grains produced by $s_u\ s_u\ W_x\ w_x$ plants which places these gametophytes at such a striking disadvantage as compared with the alternative non-waxy class. One would look first, however, for a difference in amount or kind of reserve food or inability to utilize these materials during early development of the pollen tube.

It has been shown that the composition of the pistillate parent with respect to the sugary gene does not influence the waxy ratio obtained. Whatever the nature of the differential action is, it appears to be worked out independently of any similar or complementary condition in the style of the plant. This, in itself, suggests that the difference in ability of the two classes of gametophytes to reach the embryo sac is not occasioned by difficulty in the one case in utilizing the reserves in the style but rather in using those contributed by the pollen. The chief food materials in the style of maize are sugars, no starch being present; the pollen on the other hand contains considerable amounts of starch. So far as carbohydrate metabolism is concerned we may recognize two stages in the development of the pollen tube; during the first the needs of the growing tube are met by the pollen reserves, while subsequent elongation is dependent upon supplies from the style. No observations have been made on maize pollen tubes but extensive investigations with other species show that the first period is a relatively short one; the pollen reserves are quickly consumed even in the presence of an abundance of sugar; in the plant the gametophyte must then become parasitic on the stylar tissue. So rapidly are the starch and fat reserves broken down in the initial stages of elongation that, in the case of maize, they probably serve for not more than one centimeter of pollen tube growth and perhaps less.

Further important evidence relating to the stage at which the differential development of $s_u\ W_x$ and $s_u\ w_x$ gametophyte arising from $s_u\ s_u\ W_x\ w_x$ plants occurs is afforded by results on the distribution of waxy and non-waxy seeds on the upper and lower halves of the ear. This evidence is not being given in detail at present but will be forthcoming when some supplementary experiments are completed. It may be said now, however, that following the cross $s_u\ s_u\ w_x\ w_x\ ♀ \times s_u\ s_u\ W_x\ w_x\ ♂$ the ratio of waxy seeds in the lower half of the ear is not significantly different from that in the upper half. This definitely rules out an explanation of the large discrepancy in the waxy ratio on the basis of a *constant* differential rate of pollen tube growth.

While direct evidence is wanting, circumstances favor the view that in the initial stages of development of the male gametophytes from $s_u\ s_u\ W_x\ w_x$ plants in the style the waxy tubes grow at a much lower rate than the alternative class; this relation probably obtains as long as the pollen tubes are dependent upon their own reserves. Thereafter the two classes of gametophytes develop at the same rate. One might compare the situation to two groups of bodies one of which passes a sieve more readily than the other; but, having gone through, both fall with the same speed.

SUMMARY

1. Maize plants heterozygous for the waxy factor and homozygous for the sugary gene ($s_u\ s_u\ W_x\ w_x$) give significantly lower ratios of waxy seeds on self-pollination than do $S_u\ S_u\ W_x\ w_x$ individuals. The differences in the waxy ratios given by comparable non-sugary and sugary plants in three distinct stocks were 5.21 ± 0.70 percent, 2.73 ± 0.43 percent and 11.52 ± 0.97 percent respectively.

2. The sugary gene in homozygous condition in the pistillate parent alone does not alter the waxy ratio. The combinations $s_u\ s_u\ w_x\ w_x$ ♀ $\times S_u\ S_u\ W_x\ w_x$ ♂ and $S_u\ S_u\ w_x\ w_x$ ♀ $\times S_u\ S_u\ W_x\ w_x$ ♂ both give a small deficiency of waxy seeds. The crosses $s_u\ s_u\ w_x\ w_x$ ♀ $\times s_u\ s_u\ W_x\ w_x$ ♂ and $S_u\ S_u\ w_x\ w_x$ ♀ $\times s_u\ s_u\ W_x\ w_x$ ♂ are alike in showing a large discrepancy in the waxy ratio.

3. In backcrosses of $s_u\ s_u\ W_x\ w_x$ plants to the homozygous waxy type the deficiency in the waxy class is roughly 15 percent; comparable non-sugary plants used as pollen parents in the same combination give a deviation from Mendelian expectation in the same direction of about 2 percent.

4. Tests with three stocks distinct in origin show that the waxy ratio is consistently low when the pollen parent is homozygous for the sugary gene indicating that the differentiating agent is the sugary factor itself and not some factor associated with it. In a given stock, moreover, the low waxy ratios follow the sugary gene with considerable regularity.

5. Different sugary lines vary in their norms for proportion of waxy seeds depending, presumably, upon the character of the residual inheritance.

6. As shown by the somewhat different behavior of one stock tested in two different seasons environmental circumstances may also influence the waxy ratio. In 1924, the $S_u\ s_u\ W_x\ w_x$ plants in this stock gave 24.82 ± 0.68 percent waxy seeds and the $s_u\ s_u\ W_x\ w_x$ plants 18.84 ± 0.26 percent on self-pollination. The corresponding values for duplicate progenies in 1925 were 22.88 ± 0.53 percent and 21.08 ± 0.43 percent.

7. When the double heterozygote, $S_u\ s_u\ W_x\ w_x$, is self-pollinated the proportion of waxy seeds in the sugary class is not significantly different from that in the non-sugary class. The waxy ratio in such cases approximates very closely that given on selfing $S_u\ S_u\ W_x\ w_x$ plants.

8. Following the combination $s_u\ s_u\ w_x\ w_x\ ♀ \times S_u\ s_u\ W_x\ w_x\ ♂$ the proportion of waxy seeds in the non-sugary class was 47.60 percent and in the sugary class, 48.41 percent, in a total population of 8146 grains on 21 ears. The difference is not statistically significant.

9. It is concluded that the large deficiency in the waxy class results only when the waxy heterozygote used as the pollen parent is homozygous for the sugary gene; the sugary gene in heterozygous condition in the staminate individual does not lower the waxy ratio.

10. Since the composition of the pistillate parent with respect to the sugary gene does not influence the waxy ratio obtained it would appear that the sugary gene in homozygous condition exerts a differential action on waxy and non-waxy pollen only, which is worked out independently of any similar or complementary condition in the style of the plant.

11. The hypothesis is advanced that the cytoplasm contributed by the sporophyte to the microspores is different in sugary and non-sugary plants; and that for some reason not at present known waxy gametophytes receiving sugary-type cytoplasm are handicapped in development.

12. As to the immediate cause of the deficiency of waxy seeds it is suggested that the two classes of male gametophytes, waxy and non-waxy, from sugary homozygotes grow in the style at different rates during the period in which they are dependent upon the pollen reserves and thereafter at the same rate.

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THE CHROMOSOME CONSTITUTION OF GATES' 'NON-DISJUNCTION' (*v-o*) MICE[1]*

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INTRODUCTION

In a recent paper in this journal Professor W. H. GATES has presented the genetic evidence which led him to interpret the appearance of a waltzing mouse in the F_1 generation (in crosses between pure normal and pure Japanese waltzing mice) as being due to the loss (through non-disjunction) of the chromosome carrying the normal allelomorph of the waltzing gene. For the detailed history of this exceptional F_1 individual and the genetic evidence upon which this conclusion is founded the reader is referred to GATES (1927), "A Case of Non-Disjunction in the Mouse." Very briefly the situation is as follows: The normal gait is completely dominant to the waltzing factor and in crosses between pure normal and pure Japanese waltzing mice all of the offspring (of which there were several hundred) were normal except one female (No. 1913) which was a waltzer. In subsequent breeding tests this female showed the entire absence of the normal gene and GATES concluded either that the whole chromosome carrying the normal gene had been lost (non-disjunction), or that portion of the chromosome which carried the normal gene had been lost (loss of a part of a chromosome). Genetically the two types would be indistinguishable, in the absence of a linkage between the waltzing and any other known gene.

[1] Contribution No. 204 from the Department of Zoology, UNIVERSITY of TEXAS.

* The GALTON AND WENDEL MEMORIAL FUND contributes the accompanying plates.

Knowing that the cytology of the normal mouse was being studied at this institution, Professor GATES very kindly offered to send me a few extracted (*v-o*) individuals for study. I wish to make due acknowledgment and to express my thanks to Professor GATES for the opportunity of studying the *v-o* males.

MATERIAL AND METHODS

The testes of two *v-o* males have been examined.[2] Male No. 4717 was the offspring of the original "non-disjunction" female (No. 1913) with an extracted *V-o* male. The offspring of this cross consisted of five normal and two waltzers, one of the latter being male No. 4717. The second male (No. 5347) was obtained by mating an extracted *V-o* female with a pure waltzing (*v-v*) male.

The chromosome constitution of normal mice has been a subject of study at this laboratory for more than a year, and during this time a large number of normal mice have been carefully examined.

In addition to normal and *v-o* mice the testes of one pure Japanese waltzing mouse has been studied.

In all cases the technique employed was the same that I have used in my earlier mammalian chromosome studies (PAINTER 1925). The mouse testis lends itself to preservation with the methods employed and, as the figures will show, the testes of the *v-o* males were especially well preserved.

THE CHROMOSOMES OF NORMAL MALE MICE

Work done at this laboratory (Cox 1926) has shown that the normal male mouse has 40 chromosomes (figure 1). When these are carefully examined, especially in serial alinement (figure 29), we note that three chromosomes are somewhat smaller than the rest. Of these the two smallest (labelled "s") constitute a pair while the third which is a little larger is the Y sex chromosome. The remaining chromosomes form a fairly well graded series, the X sex chromosome being one of the medium sized elements. Cox has shown that the chromosomes of one of the largest pairs often show a tendency to split early in metaphase plate stages so that they may appear unduly thick; this is not seen, however, in the cell illustrated in figure 29, but may be observed in figures 31 and 32.

[2] GATES represents normal and waltzing mice respectively by *VV*, and *v-v*. His original 'non-disjunction female' No. 1913 he designated by *v-o*, the *o* representing the absence of a gene. By appropriate matings he has been able to produce both *V-o* and *v-o* individuals, but such animals show a low vitality. He has never been able to produce *o-o* mice.

The haploid chromosome number is 20 including a typical mammalian X-Y sex chromosome complex. A side view of the latter components together with the s and a few associated tetrads is given in figure 2. Here again we note that the s chromosomes are a trifle smaller than the Y; this size relationship has proved to be very constant in all the mice I have studied, and it thus gives us a convenient standard of measure for the other chromosomes. Ordinarily the X and Y chromosomes do not segregate to opposite poles before the other chromosomes divide. In figure 33 a line-up of the haploid elements is given; this figure will be discussed in more detail later on.

THE CHROMOSOMES OF JAPANESE WALTZING MICE

Although the desirability of studying the chromosomes of pure Japanese waltzers did not appear until after the *v-o* males had been examined, the main facts will be presented here. The one Japanese waltzer studied has 40 chromosomes (figs. 3 and 4). It will be noted that the s and Y chromosomes can be identified readily and that they have the same proportionate sizes as in the normal mouse. In the first maturation division it was observed that the sex chromosomes show a marked tendency to segregate early to the poles of the spindle when they are very conspicuous. In other respects no deviation from normal conditions was observed in the individual studied.

THE CHROMOSOMES OF *v-o* MALES

Spermatogonia

In my first preliminary counts on dividing spermatogonia I was able to find only 39 chromosomes, but more careful and extended study of a number of extremely favorable cells showed that 40 chromosomes is the true diploid number for both males (figures 5 to 8). On returning to the cells on which the first counts had been made in order to find out the source for the discrepancy in number, I found that when I counted 39 chromosomes I had failed to observe one very small chromosome, which is always present in cells with 40 chromosomes. Subsequently this small element was discovered in practically all of the cells which at first had shown only 39 chromosomes. When the individual chromosomes of spermatogonia are carefully examined, either in equatorial plate view (figures 5 to 8) or better in alinements in which each chromosome is drawn separately (figures 30 to 32), it becomes apparent that both s chromosomes and the Y sex chromosome are present in *v-o* males, and show normal size relations. The small chromosome, which I have labelled

LEGEND FOR PLATE 1

All figures on this and succeeding plates represent a magnification of approximately 3700 diameters as reproduced.

FIGURE 1.—The spermatogonial chromosomes of a normal mouse.

FIGURE 2.—A few chromosomes of a first maturation spindle of a normal mouse showing the X-Y sex chromosomes and the s complex.

FIGURES 3, 4.—Spermatogonial chromosomes of a pure Japanese waltzing mouse.

FIGURES 5, 6, 7, 8.—Spermatogonial chromosomes of two *v-o* males, showing 40 chromosomes including the fragment q_1.

FIGURES 9, 10.—First maturation spindles from *v-o* mice showing the position assumed by the sex chromosomes. Only a few other elements are shown.

FIGURES 11, 12.—Equatorial plate views of first maturation division taken from *v-o* mice showing 20 chromosomes.

1
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10
11
12

"q_1" for reasons given later, is typically smaller than either s chromosome, and there is no other chromosome of the same size to be found in the cell with it. The size of the q_1 chromosome varies somewhat depending on the degree to which differentiation of the stain has been carried. When the cell is heavily stained, as in figure 8, the q_1 approaches the s chromosome in size, but in less deeply stained cells (figures 5 to 7) it is markedly smaller than the s elements. Since the two s chromosomes are the smallest elements found in either normal (figures 1 and 29) or waltzing mice (figures 3 and 4), it is evident that the q_1 chromosome of *v-o* mice had no morphological homologue in either of these races. It must therefore be either a fragment of one of the chromosomes larger than the s and Y elements, or simply a supernumerary chromosome introduced by chance into this stock.

A mating up of the spermatogonial chromosomes, and their arrangement in a serial alinement shows (figures 30 to 32) that one of the smaller chromosomes, lying between the limits of the "p" and the "s" pairs (using Cox's terminology) lacks a mate of like size. Assuming that the q_1 chromosome is a fragment of a normal chromosome, its homologue must lie in this region. In the alinements I have placed the q_1 chromosome with the q element, my reason for doing this, however, is based more on primary spermatocyte than on spermatogonial evidence. It is clear that considering spermatogonia alone one would be unable to say whether the q_1 was a fragment of a normal chromosome or a supernumerary.

First maturation period

During this period GATES' *v-o* mice show two unusual features not observed in normal mouse spermatogenesis, and for a considerable time I was misled with regard to the correct interpretation of the observed facts. A very careful study of diakinesis stages together with an examination of the waltzing mouse testes gave the necessary clues for clearing up the situation. For the sake of clarity and brevity these usual features will be discussed and explained before a detailed account of this period is given.

The very first tubule examined showed a first maturation spindle in which a large and small chromosome lay at opposite poles (figure 9). Near this was another cell in which, judging from size relations, the same elements were passing undivided to the same pole (figure 10). Numerous instances were noted in both males in which one of these two conditions obtained. There was no visible connection between these two aberrant chromosomes and since they did not divide and their distribution to the

same pole or to opposite poles appeared to be a matter of chance, it seemed obvious that we had at this period two chromosomes which behaved as univalent elements. And furthermore, since a small chromosome occurs in spermatogonia which had not been observed in normal mice, it looked as if the *v-o* males carried a supernumerary chromosome and a larger univalent chromosome which presumably had arisen through non-disjunction, as GATES had concluded. On the other hand, it was soon realized that the size relations of these two aberrant chromosomes was about the same as that of the X and Y sex chromosomes of normal mice and that while in spermatogonia the q_1 chromosome was smaller than the s elements in the first maturation stage, the small aberrant chromosome was a little larger than the s components. The upshot of the whole matter turned out to be this: That as a close study of the diakinesis stage showed, the connection or association between the X and Y sex chromosomes is often very weak, and as a result they may come into the spindle without any visible connection between each other. This lack of connection seems to retard their orientation in the spindle, and if they happen to lie on one side, as in figure 10, one gains the impression that they are passing together to one pole.

A second unusual feature of *v-o* males is the large number of degenerating first spermatocytes. GATES has emphasized the frail nature of these *v-o* mice, and as the testes were preserved a day or two after they had been received from a long railway journey, there can be no doubt that the hardships of the journey are responsible, in part at least, for the degeneration observed. Degenerating cells are characterized by a rounding up of the cell, and a darkening of the cytoplasm (with iron haematoxylin). At the same time the chromosomes seem to shrink or separate so that they are very favorable for counting. The exceptional feature which makes it necessary to eliminate such cells from consideration is that the chromatoid body, which in normal cells is extremely small and lightly staining, takes the stain deeply and may become quite large. In well differentiated material, the walls of the vacuole in which the chromatoid body appears to lie can be readily detected, but in deeply stained cells this chromatoid body looks like a small chromosome, and the writer mistook it for a time for the q_1 element.

Once the erratic behavior of the X and Y sex chromosomes was understood, and degenerating spermatocytes were eliminated from consideration, an analysis of observations on the first maturation period proved easy and very illuminating.

In the diakinesis stages, when the haploid chromosomes appear with ring-like and other characteristic shapes, we have the first opportunity of determining the make-up of the bivalent elements. If the small chromosome q_1 is a fragment of a normal chromosome, we should expect it to be associated with its homologue at this time, and to find among the tetrads an element, the two parts of which are very unequal. Needless to say, the X-Y chromosomes should also appear as a heteromorphic group, and must not be confused with the q_1 complex. In diakinesis stages I have found two heteromorphic groups, and it has proved possible to follow each of these through the first division in considerable detail as described below.

In diakinesis the X-Y complex (easily recognized because of its size relative to the s chromosome group) is found in two conditions. Usually the shorter Y is attached either to the end of the X or, as in figure 13, lying parallel to it on one side. More rarely, as in figure 15, the X and Y are entirely separated. The element which I have identified as the q_1 chromosome is associated with a chromosome some four times as large. Figure 14 is an early stage showing the relation between the larger q and the q_1 chromosome. Figure 15 is especially interesting because we have in the portion of the cell illustrated, not only the q_1 complex but the X-Y complex and the s chromosome pair. We are thus able to check up on the matter of relative sizes. The q_1 component is somewhat smaller than either s element, and in turn we note that the Y is a little larger than the s.

Reflecting the loose association of the diakinesis period we find that the X and Y chromosomes exhibit a considerable difference in their behavior in fully formed first maturation spindles. In a considerable majority of cases, these two components are attached end-to-end by a deeply staining chromatic bridge (figures 16 to 18).[3] Figures 19 to 21 are cells in which the X and Y are separate with no visible connections and represent perhaps, steps in the eventual arrangement and distribution of these elements. Figure 25 is a cell in which the sex chromosomes are entirely separated by the spindle. It seems altogether probable in the cells represented in figures 19 to 21 and 25, that there had been an entire separation of the X and Y in the diakinesis stage, similar to that shown in figure 15.

It has proved possible to follow the q_1 complex through this division often in the same cells showing the sex chromosomes. Figure 20 shows the q_1 element beginning to separate from its mate, and in figures 21 to 24

[3] The variations in size of the sex chromosomes and of other elements in the figures are due in part to differences in the degree of condensation, and in part to differences in staining. Differences in preservation may also play some part here.

LEGEND FOR PLATE 2

FIGURES 13, 14, 15.—Diakinesis stages taken from *v-o* mice to show the behavior of the sex chromosomes and of the q-q_1 complex. Not all of the elements are drawn in.

FIGURES 16,17,18.—Detailed drawings of sex chromosomes in *v-o* mice early in the first division.

FIGURE 19.—Side view of a first maturation spindle from a *v-o* mouse showing the sex chromosomes unconnected, but lying close together.

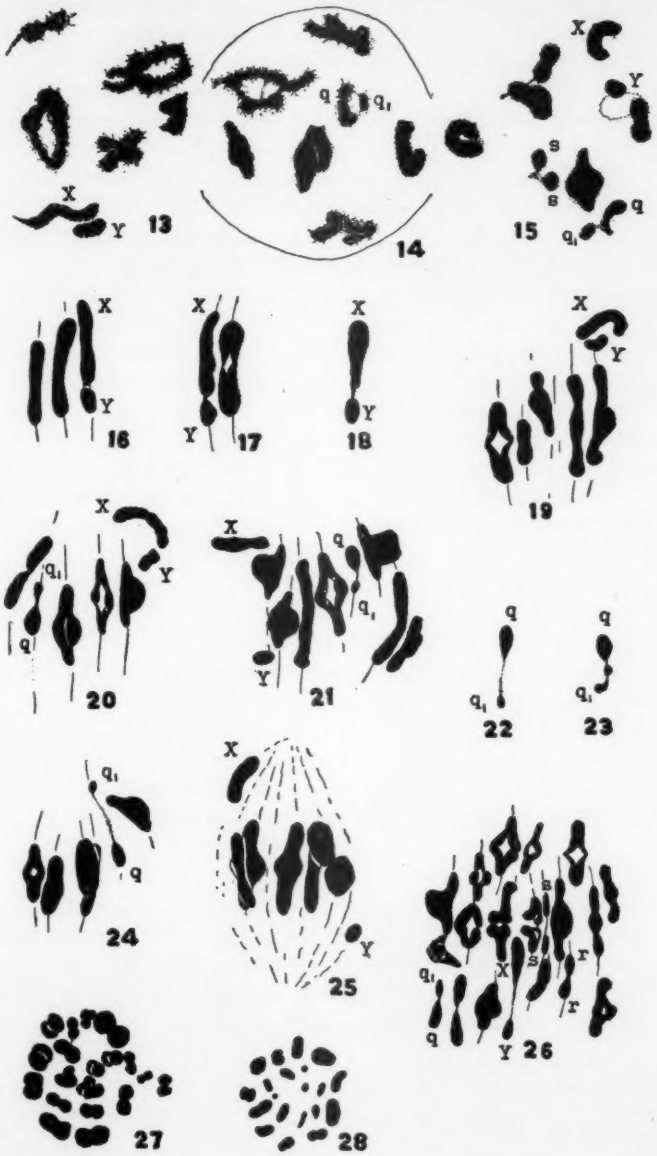
FIGURES 20, 21.—Side views of spindles from *v-o* mice showing both the sex chromosomes and the q-q_1 complex.

FIGURES 22, 23, 24.—Detailed drawings of the q-q_1 complex from different cells of *v-o* mice.

FIGURE 25.—First maturation spindle from a *v-o* mouse showing the separation of the sex chromosomes.

FIGURE 26.—A "spindle dissection" of a first maturation spindle from a *v-o* mouse showing the morphology of the 20 haploid elements.

FIGURES 27, 28.—Second spermatocyte chromosomes showing 20 chromosomes from *v-o* mice.


X
Y
13
q
q1
14
X
Y
s
s
q
15
q1
X
Y
16
X
Y
17
X
Y
18
X
Y
19
X
q1
Y
q
20
X
q
q1
Y
21
q
q1
22
q
q1
23
q1
q
24
X
Y
25
q1
X
s
s
r
r
q
Y
26
27
28

other views and stages of separation are shown. As the two components draw apart it is not unusual to see a distinct knot in the chromatic bridge (figure 23) and a little later (figure 24) the chromatic bridge is much heavier than before suggesting that this knot has contributed to the bridge. I have gained the impression that as the q and q_1 chromosomes go apart in this division the heavy bridge goes along with the q_1 element.

Figure 26 is a "spindle dissection"[4] of one of those rare cases in which all the elements of the first maturation spindle can be recognized in one cell and in figure 34 these are lined up in the approximate order of their size. From the standpoint of the present paper the most interesting elements are the sex chromosomes, the q_1 and the s complexes. Certain phases of this figure will be taken up below.

Primary spermatocytes have 20 chromosomes. In many cells the sex chromosomes lie well outside of the general circle of autosomes, as in figures 11 and 12. If the X and Y happen to be separated the count may run to 21, or if the X and Y have already segregated to opposite poles, we may find only 19 elements. The higher and lower counts are obviously correlated with the erratic behavior of the sex chromosomes.

All the evidence of the first maturation division points to the segregation of the q and q_1 elements to opposite poles of the cell, just as in the case of the sex chromosomes.

Second maturation division

It has proved difficult to reach any definite conclusion regarding the behavior of the q_1 chromosome during this division. Twenty chromosomes are found in second spermatocyte cells (figures 27 to 28), but the small size of all the chromosomes makes it difficult to identify the q_1 element. In side views of spindles I have seen no evidence which would indicate that this was other than an equation division for all of the chromosomes.

DISCUSSION

In interpreting his genetic results, as I have stated above, GATES concluded either that a whole chromosome had been lost, or that a part of a chromosome was absent. In the former event we should expect *v-o* mice to show 39 chromosomes, but if it were a case of a loss of a part of a chromosome, there should be 40 chromosomes, and the detection of the

[4] In "spindle dissections," as I have used the term in various papers, the individual chromosomes are drawn more or less out of place so that their form may be clearly seen. In reality they lie in a normal spindle with much overlapping, but by focusing up and down on the semi-transparent elements the shape of the lower lying members can be accurately determined.

loss cytologically would be dependent upon how large a piece of the chromosome was gone. The non-viable character of *v-o* mice and GATES' failure to obtain *o-o* individuals both suggest that the loss may be extensive in character.

A cytological study of *v-o* mice has shown that they carry the full normal diploid number of 40 chromosomes. At first this would seem to eliminate the possibility of the loss of a whole chromosome, but this would not be the case if the *v-o* stock should happen to carry a supernumerary chromosome. As a matter of fact, in my preliminary study when I confused the deeply staining chromatoid body of degenerating cells with a true chromosome, I suspected the presence of a supernumerary body, but later observations gave no valid evidence for this.

With the elimination of the loss of an entire chromosome we must now consider if we have critical evidence for the loss of a part of a chromosome. The answer is clearly in the affirmative. Both *v-o* males show a very small chromosome in their spermatogonia which is not present in either normal or waltzing mice, and in the first maturation division this fragment is actually observed paired up with a chromosome four or more times as large. This is just the sort of behavior we should expect to find in case the q_1 chromosome was a fragment of one of the chromosomes of the normal mouse complex, and is inexplicable should we try to interpret the q_1 as a supernumerary. It is clear that a loss of part of a chromosome would fully explain GATES' exceptional mouse as well as non-disjunction. We only need to assume that the normal chromosome q carries the factor for waltzing and that the fragment q_1 lacks this gene (and many others no doubt).

The cytological study of *v-o* mice has thus made a distinct contribution to our knowledge of the genetic consequences of aberrant chromosome behavior. For it is the only case on record in which the absence of a part of one chromosome (cytologically determined) is accompanied by an absence of a gene (genetically determined) although the fundamental principle involved is familiar to us in non-disjunction and is suggested in cases of so-called "chromosome deficiency." Cytologists have known for a long time of animals in which one autosome was markedly larger than its synaptic mate (see WILSON, 1925, p. 931), but such cases have not been linked-up with the absence of genetic characters. On the genetic side, cases of "chromosome deficiency" have been reported which were not accompanied by any visible loss of chromatin. In *v-o* mice both cytological and genetical conditions are fulfilled. At first sight, one would describe this as a case of "chromosome deficiency," which literally

it is of course, but unfortunately this term has been used by cytologists and geneticists to mean slightly different things. In cytology it has meant the absence of a part of a chromosome, but BRIDGES who first employed the term in genetics applied it to cases where a gene or a group of genes were genetically absent, but it was thought that this might be due to an inactivation of genes, since no morphological deficiency could be observed in the chromosome involved. For this reason the writer proposes the term "chromomere deletion" to cover cases such as GATES' *v-o* mice, meaning what this term literally implies, the loss of a segment (large or small) of a chromosome from the cell. It may well be that cases of "deficiency" in the genetic sense are conditioned, in most instances, on chromomere deletion.

From a cytological point of view the behavior of the chromosome fragment in the mouse is like that of similar structures in Orthoptera. And it is interesting to note that in the mouse we have evidence of a random segregation of the chromosome fragment, using the sex chromosomes as a criterion, just as CAROTHERS (1913) so clearly described in the grasshopper Brachystola. In figures 20 and 26, the fragment q_1 is passing with the X chromosome to one pole, while in figure 21 it is going to the same pole as the Y chromosome.

From a theoretical standpoint, perhaps the most interesting feature of *v-o* mice is that they afford us the first opportunity of locating a definite gene in a definite chromosome in mammals. Because of this theoretical interest a great deal of study has been given to the exact identification of the snyaptic mate of the chromosome fragment q_1. The first attempt at this was made in spermatogonial cells in which the individual chromosomes of the cell were copied separately and then matched up according to relative size and to a slight extent shape. This mating was checked under the microscope so as to eliminate, as far as possible, any error due to foreshortening. The results of these alinements are given in figures 30 to 32, and allow us to locate the mate of q_1 among some one of the smaller chromosomes. In most cases it appears to fit in best with the q chromosome, but the method due to unavoidable sources of error is not sufficiently exact to allow us to more than approximate the location of the q_1 element on spermatogonial evidence.

The first maturation division has given much more satisfactory evidence regarding the synaptic mate of q_1. In figure 34 the bivalent chromosomes of a complete first maturation spindle are shown individually. Beginning at the end with the small chromosomes, we note the smallest or s chromosome pair. A trifle larger is the r pair, and next to it in size is the q chromo-

LEGEND FOR PLATE 3

FIGURE 29.—A chromosome alinement of the spermatogonial chromosomes of a normal mouse taken from figure 1.

FIGURES 30, 31, 32.—Chromosome alinements from *v-o* mice based on figures 5 to 7 respectively.

FIGURE 33.—The haploid elements of a normal mouse.

FIGURE 34.—The haploid elements of a *v-o* mouse, based on figure 26.

29
30
31
32
33
34
pp qq rr ss X Y
A B C D E F G H I J K L M N O P Q R S XY

some to which the q_1 is attached. Using the s chromosome as a standard of measure, I have checked up repeatedly this identification of the mate of q_1 and I am convinced that the q element is its homologue.

There is one additional reason for identifying the chromosome fragment as belonging to the q pair. In normal mice as Cox (l.c.) has shown, the q chromosome typically divides much earlier than the rest of the elements. In *v-o* males I have not observed the presence of this precocious pair, except in so far as the q and q_1 elements tend to separate early (figures 20 to 24). Furthermore, the erratic behavior of the q elements in normal mouse spermatogenesis would seem to make them particularly liable to an accident during maturation. All of the foregoing considerations have led me to the conclusion that the initial loss of chromatin occurred in a q chromosome.

There is one additional feature of the present study which deserves mention, and that is the unusual behavior of the sex chromosomes. Why should the sex chromosomes exhibit, in some cells, such a loose association? Is it not possible that this would lead to an irregular distribution of the sex chromosomes, with the attendant production of sexually abnormal individuals? These two questions can be answered only in a provisional way.

Since normal mice only occasionally show the early separation of the sex chromosomes, while in the one Japanese waltzer examined this is the rule, it seems probable that the unusual behavior of the sex chromosomes has been brought into the *v-o* mice from the waltzing strain. In this connection it should be mentioned that recently GATES (1926) has shown that the Japanese waltzing mice are probably derived from a species different from the common fancy mouse. This might account for the loose association between the sex elements in crosses. With regard to the second question, it seems inevitable that, if the sex chromosomes separate entirely before they enter the first maturation spindle, then there is a chance that the X and Y would pass together to one pole, and thus give the basis for sexual abnormalities. However, my observations indicate that spermatocytes showing this behavior are not a large class numerically, although they are very conspicuous, and hence the chance of a sperm carrying both the X and Y sex chromosomes fertilizing an egg is not very great.

SUMMARY

1. A cytological examination of two of GATES' *v-o* mice shows that each has the full diploid number of 40 chromosomes.

2. A close examination of the individual spermatogonial chromosomes reveals that in *v-o* mice there is a small chromosome fragment which is smaller than any element in either normal or waltzing mice.

3. Evidence from the first maturation division indicates that the chromosome fragment is a part of the q chromosome with which it unites in synapsis.

4. In the first maturation division the fragment segregates from its homologue so that mature spermatozoa will carry either the normal q element or the fragment q_1.

5. GATES' original waltzing female (*v-o*) is to be explained, therefore, on the basis of an absence of a part of a chromosome rather than on the basis of the absence of an entire chromosome.

6. Since the term "chromosome deficiency" has come to have two slightly different meanings in cytology and genetics, the writer suggests the term "chromomere deletion" to cover cases such as GATES' *v-o* mice where there is a visible absence of a part of a chromosome.

7. The *v-o* mice allow us for the first time in any mammal to locate a definite gene in a definite chromosome.

8. The erratic behavior of the X-Y sex chromosome complex during the first maturation division has been described.

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